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5879

A Simple Culture Medium for Endamoeba Histolytica.

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As contrasted with various media generally used in the cultivation of *Endamoeba histolytica*, the proposed medium is simple in its composition. It consists of sterile nutrient broth and a mixture of starch and charcoal. The nutrient broth is adjusted to pH 7.0 and is left in an incubator until used, or warmed to about body temperature at the time of inoculation of a suspected material. To the above medium is added rice starch and animal charcoal mixture (in the proportion of 2:1 by volume) briefly designated as S.C. mixture in this paper. A small amount of the S.C. mixture is loosely placed in a small tube and sterilized by dry heat at 180°C. for 45 minutes. Rice starch, according to Dobell and Laidlaw,[1] provides the amoebae with a definite source of assimilable carbohydrates and also inhibits the growth of *Blastocystis* frequently found in feces. Animal charcoal is used to adsorb ammonia and hydrogen sulphide present in the culture, thus reducing their deleterious effects upon the amoebae, while calcium phosphate contained therein helps to stimulate the metabolic activities of the organisms.

Formed stool is thoroughly mixed by means of a sterile glass rod in order to obtain a uniform distribution of the cysts. Liquid stool should first be sedimented, and the supernatant fluid decanted. Washed cysts are then prepared by filtration and sedimentation of the stool, followed by repeated washings with centrifuging. Cysts in freshly sedimented stools grow almost as well as those obtained in the stools left standing for several hours at room temperature or in a refrigerator.

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[1] Dobell, C., and Laidlaw, P. P., *Parasitol.*, 1926, **18**, 283.

Procedure. A small amount of washed cysts (0.1 cc. or less dependent upon the number of cysts present) is introduced into 8 cc. of the broth, thoroughly mixed by shaking and a 5 mm. loopful of the S.C. mixture is added. The tube is now incubated at 37°C., the optimum incubation period being 24-48 hours. By means of a 1 cc. pipette with a wide terminal opening, the sediment is at first gently scraped off from the bottom of the tube, and then about 0.1 cc. is withdrawn. This is spread on a warm clean slide, covered with a cover glass, and examined by means of a 10 X eye piece and 16 mm. dry objective preferably either on a warm stage or in a warm chamber. The addition of a small drop of 0.1% neutral red will facilitate the examination greatly, as it gives the amoebae a pinkish refractive tinge as contrasted with other objects in the microscopic fields. Care should be taken to guard against draught at any stage of the procedure, as this may cause the amoebae to round up and die.

By the use of this medium, the amoebae are often seen at various stages of development. The medium also seems to be practically a specific one for *E. histolytica,* as other endamoebae showed none or only an extremely poor growth in it. The rate of growth of the amoebae in this medium is very striking. Thus, by the introduction of 80 cysts, the number of trophozoites was calculated to be 21,200 at 48 hours incubation in one instance, while in the others, 40 cysts gave rise to 17,900 and 72 cysts to 12,200 trophozoites.

Furthermore, this medium may be employed very effectively by covering the coagulated egg slant of Boeck and Drbohlav,[2] or that of Dorset.[3]

Immunological studies are in progress with this culture material as an antigen.

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[2] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, **5**, 371.

[3] Dorset, M., *Am. Med.*, 1902, **3**, 555.

5880

Distribution of Mineral Ash in Striated Muscle Cells.

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This report is a brief account of one phase of a study of the distribution of inorganic salts, in various tissues and cell types, as revealed by the technique of microincineration. The method of incinerating thin sections of tissue without disturbing the topography of the mineral components was suggested by Liesegang,[1] but its advantages were never realized until the researches of Policard.[2] Until recently the technique has been largely a gross histologic one because of inherent difficulties in making observations with the higher powers of the microscope. However, it was discovered by the writer[3, 4, 5] that the use of the dark field condenser of Zeiss removed to a large extent these difficulties and rendered it possible to follow through even the changes of the fixed minerals of the nucleus during mitosis.

It is necessary to use a fixative which neither removes nor increases the inorganic elements of the tissue. Absolute alcohol is best adapted for this purpose, but gives poor fixation even under the most favorable circumstances. It was found after some experimentation that a mixture of 9 parts of absolute alcohol to 1 part of neutral formalin (Will Corporation) gives rather good cytologic detail and yet, for all practical purposes, does not change the original inorganic constitution of the tissue. After fixation for 24 hours the tissue is passed through several changes of absolute alcohol to complete the dehydration. The specimens are then cleared in xylol, embedded in paraffin and cut serially at 4 microns. Alternate sections are mounted according to the usual technique and stained with haematoxylin and eosin. The intervening sections of the series are mounted on glass slides with liquid petrolatum as a medium in which to spread the tissue. Sections prepared in this manner are incinerated in an open electric quartz tube furnace. The highest temperature of incineration, approximately 650°C., is reached by slow steps covering from 25 to 40 minutes. When the incineration is completed the slide is cooled slowly, and carefully covered with a number 0 coverglass, the edges of which are then sealed with melted paraffin.

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[1] Liesegang, R. E., *Biochem. Z.*, 1910, **28**, 413.

[2] Policard, A., *Protoplasma*, 1929, **7**, 464.

[3] Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1073.

[4] Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1323.

[5] Scott, G. H., *Bull. Hist. app.*, 1930 **7**, 251.

The preparation may be examined with the darkfield condenser (Zeiss Cardioid) and oil immersion objectives. Under favorable conditions photographs can be taken with a Zeiss achromatic objective 90 N.A. 1.25 (equipped with an iris diaphragm) and a 10x ocular (see figure 1).

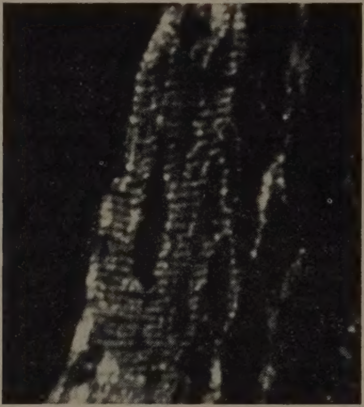

FIG. 1.

Photograph of incinerated longitudinal section of rectus muscle of cat. Zeiss achromatic 90 N.A. 1.25 (with iris diaphragm) 10x ocular and Zeiss Cardioid condenser.

The inorganic salts which are not volatilized at 650°C. retain the same topographical distribution as in the fixed tissue. The residue is then essentially a mineral skeleton of the cells free from organic materials. With some practice tissues may be identified quite as well by their ash deposits as by their appearance when prepared by the ordinary histologic methods. Iron can be identified by its color since it forms iron oxide under the influence of the incineration. Calcium is in all probability present as an oxide. It is difficult to say in what form the remaining salts are found. Silicates can be detected by their birefringence when viewed with polarized light.

When skeletal and cardiac muscles are subjected to incineration, the resulting ash is arranged in a distinctly characteristic manner. The striations are preserved with startling clearness (Fig. 1). Crossing the individual fiber may be seen stripes which are composed of small deposits of whitish ash, in some places tinged with the yellowish red which denotes the presence of iron. Alternating with these stripes of ash deposit are areas almost clear of mineral residue. Comparisons with the adjacent stained serial sections show that these cross bands of heavy ash deposit correspond very well with the Q, or anisotropic discs seen in ordinary preparations. The striations which are ash-free are in all probability the remains of the J, or isotropic bands.

In favorable preparations a fine line of ash may be seen traversing the J band. This disc corresponds to the Z, or intermediate band of the J striation. In some instances a differentiation of the Q band of ash can be distinguished. When this happens there is a definite row of twin deposits of mineral material. This appearance is especially striking in cardiac muscle. In skeletal muscle the sarcolemma shows as quite a distinct line of whitish ash in which there are some deposits of silicates. Cardiac muscle does not present as sharp an outline of each muscle fiber. In many instances it is possible to find isolated myofibrils with their characteristic bands of ash-bearing and of ash-free substances.

Thus far it has not been possible to distinguish any deposits of ash similar in form to the intercalated discs of cardiac muscle. Structures which are in all probability motor end plates show as a fine, heterogenous mass of mineral material with little or no orientation of the deposit. The nuclear ash of the skeletal muscle cell shows as a dense deposit at the periphery, while in heart muscle it is found in the center of the fiber. Simple chemical tests indicate that the nuclear ash is largely calcium. No striking differentiation of the ash deposit of the Purkinje fibers has yet been noted.

5881

Evidence of Possible Occurrence of Anaphylactic Phenomena in Poliomyelitis Immune Monkeys.*

WM. B. BREBNER. (Introduced by E. V. Cowdry.)

From the Anatomical Laboratory, Washington University.

In connection with a series of experiments whereby monkeys are injected intrasplenically with poliomyelitis virus and subsequently develop an appreciable immunity, my attention has been drawn to a phenomenon which may be evidence of an anaphylactic reaction.

Monkeys convalescent from an experimental infection and there-

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* Supported by a grant from the International Committee for the Study of Infantile Paralysis.

fore immune, monkeys experimentally immunized, and a small group of monkeys proving naturally immune, if injected intrasplenically or intraperitoneally with virus will invariably die. Death may be either sudden while injection is still in progress or may follow the injection by some days when the intervening period is characterized by increasing weakness. Sudden death is usually seen when the virus is administered intrasplenically and the lingering one is typical of the intraperitoneal administration.

This reaction has only been observed after 47 days or more have elapsed since immunization was commenced or the abortive infection occurred. Immediate death has always taken place while the animal was under ether anesthesia. Respirations become rapid and shallow, soon inspiration becomes spasmodic and expiration passive and prolonged. The interval between inspiratory efforts increases, and finally efforts cease. Post mortem reveals that the lungs are collapsed and the heart is still beating. Microscopically in these cases one finds mucosal hemorrhages in the gut, a degree of central necrosis in the liver and hemorrhage into the medulla of the suprerenal. The spleen shows the same changes as do all monkeys which have received polio virus.

The first group consists of 3 monkeys. The first two had recovered from an infection with the well-known Flexner "mixed-virus", receiving an intracerebral dose of 0.01 cc. and 0.3 cc. respectively of a Berkefeld 'N' filtrate of a well-centrifuged 5% suspension. The third monkey had received 1 cc. of a 20% centrifuged suspension of cord material obtained from a human case. The first 2 monkeys died while receiving the intrasplenic dose, the third survived 4 days, growing progressively weaker, finally became moribund and had to be sacrificed.

The second group consists of 4 experimentally immunized monkeys. These animals had received virus intrasplenically from 72 to 200 days previously and had a slight but recognizable titre of circulating antibodies as revealed by the neutralization test. The first 3 received the second dose of virus intrasplenically and died on injection. The fourth received virus intraperitoneally and growing gradually weaker was killed after 8 days while in a moribund state.

The third group consists of 2 monkeys which were apparently naturally immune for on 2 occasions each was injected intracerebrally with potent virus and showed no signs of infection. Monkey No. 167 died immediately on receiving the intrasplenic injection, while No. 253 survived a similar injection for 4 days and finally had to be sacrificed because of extreme weakness.

In an attempt to ascertain whether this reaction was induced by virus, or the nerve tissue necessarily mixed with virus, the convalescent monkey, first group, was injected intrasplenically with a heavy suspension of normal monkey brain and cord tissue. There was no reaction at the end of 5 days so the animal was re-injected in the same fashion with virus and death was immediate. One additional monkey which had been injected with virus intrasplenically without showing detectable virucidal antibodies at any subsequent date was also injected intrasplenically with normal monkey brain and again without untoward effect.

From these experiments it would appear that this peculiar reaction is due to the interaction of the virus antigen and antibody. One other possibility exists, namely, a sensitization of the monkey to some particular protein degradation product produced in nerve tissue by virus action. Hindle[1] has described a comparable reaction in monkeys immunized to yellow fever virus and reinjected after 3 to 4 months.

If this reaction be due to virus it may provide a means of determining whether more than one strain exists in this country and abroad. When virus has been sufficiently purified certain of these reactions will be reexamined.

5882

Active Immunity to Endemic Typhus Fever as Produced by Formolized Infected Tissue.

H. A. KEMP. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology, Baylor University, College of Medicine, Dallas, Texas.

Formolized tissue *(Tunica vaginalis)* prepared according to Zinsser's method[1] and administered intraperitoneally in 4 weekly doses of 1 cc. each to non-infected guinea pigs brought about in test animals an immunity to at least 200 times enough virus to cause a scrotal reaction in 4 days. One cc. of a 1-10 saline emulsion of tissue taken at the height of reaction contained at least 200 times enough virus to cause a typical typhus reaction in the male guinea pig after 4 days.

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[1] Hindle, E., *Med. Res. Council*, 1930, **7**, 460.

[1] Zinsser, H., and Batchelder, A. P., *J. Exp. Med.*, 1930, **51**, 847.

Animals similarly tested one week after 1, 2, or 3 1 cc. doses of "vaccine" all reacted positively to the test dose. Furthermore, protection was not found to be complete in animals that had received 1 or three 1 cc. doses of "vaccine" plus 4 weeks' rest when these animals were given the test dose. Only animals receiving 3 doses of vaccine followed by 4 weeks' rest were found to have any resistance at all to the test dose, and while these animals developed no definite scrotal reactions, all of them had temperature courses typical for endemic typhus fever in the guinea pig.

To determine the duration of their resistance, 6 animals previously immunized were tested at various intervals by inoculating them intraperitoneally with 1 cc. Two of these test animals were immune to the above dose 2 months after completion of the four 1 cc. dose "vaccination", and 2 others were immune to the test dose 3 months after the same "vaccination". When tested at 4 months one of the remaining 2 animals developed a slight scrotal swelling 4 days after receiving the test dose. This swelling persisted for 3 days and was accompanied by daily temperatures of 103.2°F. to 105.0°F. The sixth animal had no scrotal swelling, but on the fifth, sixth and seventh days following the test dose its temperature was 104.2°F., 105.2°F., and 104.2°F. In the course of these and other studies in endemic typhus it was found that 68 animals which had recovered from the infection produced by transfers of the virus remained immune to the test dose for periods varying from one month to one year.

In "vaccinating" the animals all had rather marked temperature reactions during the 2 days following inoculations. The reaction was never less than an increase of 1½°, and in many instances temperatures of 104.4°F. were noted. Zinsser[2] has reported similar reactions.

Formolized tissue kept for 4 weeks at 9°C. did not protect guinea pigs against the test dose described above when administered in either 1, 2, or 3 cc. doses given 4 times at intervals of one week. Nine animals so treated, (3 in each group) were tested 2 weeks after completion of "vaccination" as in the foregoing experiments. All of these animals developed scrotal swelling and typical temperature reactions following the intraperitoneal injection of 1 cc. of a 1-10 saline emulsion of infected tunica.

The virus after preservation in glycerin and storage for 7 days at 9°C., given in 4 cc. doses of equal parts of 1-10 saline emulsion of infected tunica and glycerin failed to produce a scrotal reaction.

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[2] Zinsser, H., and Castenada, M. Ruiz, *J. Exp. Med.*, 1931, **53**, 493.

This amount of virus would be, roughly speaking, 400 times enough to cause a scrotal reaction in 4 days, or twice the test dose used in the above experiments.

Tests were made using both the glycerin and saline mixture and the tissue washed free of glycerin and taken up again in saline. In both instances test animals showed only a slight rise in temperature which occurred 4 days after inoculation and lasted only 2 days before returning to normal. In six animals so tested no temperatures higher than 103.6°F. were observed. Transfers of heart's blood, *Tunica vaginalis,* spleen and brain failed to re-establish the virus after 2 passages. Glycerolated virus preserved for 2 weeks produced no reaction at all when given under similar conditions.

It would appear that the immunity produced by inoculations of formolized tissue is not lasting, and that its production is accompanied by marked reaction to the relatively large quantities of "vaccine" substance employed. The "vaccine" retains its potency only for a short time.

The question of the nature of the "vaccine" (as to whether the virus is formol killed or formol attenuated) remains unsolved. It would seem, however, that the virus in formolized tissue would be dead since infected tissues preserved in glycerin and stored for 2 weeks at 9° C. produced no reaction at all.

5883

Serum Sickness in Offspring of Serum Injected Rabbits.

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In the rabbit, manifestations of serum sickness are apparent upon the ears 3 to 7 days after a primary injection of serum, and consist of a characteristic erythema with or without an edema. In some animals that have previously received serum there is noted upon a second injection of the serum, similar manifestation of serum disease occurring however, within a few hours to 3 days post-injection; these reactions are probably analogous to the immediate and accelerated types of serum sickness occurring in man incident to a second injection of serum.[1]

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[1] Fleisher, M. S., and Jones, L., *J. Exp. Med.*, 1931, **54**, 597.

It was of interest to determine whether or not this capacity for immediate or accelerated reaction acquired upon serum injection, was transmitted from serum-injected mother to offspring. Accordingly in a limited number of young rabbits born of serum-injected mothers an attempt was made to determine the extent of the latent period that would elapse between time of injection and the advent of serum sickness.

In a litter of 6 young animals 53 days old, offspring of rabbit injected with horse serum 50 days prior to birth of young, serum sickness as indicated by erythema and edema of the ears was observed in 2 animals within 7 hours after serum injection, in another animal serum sickness occurred on the 6th day after injection while in 3 animals no reaction was observed. The immediate reactions (those occurring within 7 hours) indicate probable transmission from serum-injected mother to offspring of potential capacity for hastened reaction upon serum injection, since similar early reactions have never been noted in more than 150 normal full-grown rabbits receiving a primary administration of serum.

In 9 young animals 53 to 129 days of age, the offspring of 4 animals which had been injected with serum 23 to 50 days prior to birth of young, serum sickness was observed only in 1 animal and at 5 days after injection.

Since immediate reactions have never been observed in normal rabbits injected with serum it may be assumed that the occurrence of immediate reactions in the 2 animals noted above, is indicative of transmission from serum-injected mother to offspring of the potential capacity for early reaction to the administration of serum.

Pacific Coast Section.

Stanford University Medical School, December 17, 1931.

5884

Effect of Sodiummonoiodoacetate upon Metabolism of Streptobacterium casei.

JOHN FIELD AND S. M. FIELD. (Introduced by E. G. Martin.)

From the Laboratories of Physiology, Stanford University.

Present evidence indicates that sodiummonoiodoacetate is a fairly specific inhibitor of the lactic acid cycle in frog's muscle and in many other tissues.[1] However, the electrical, mechanical, and thermal aspects of the first few contractions of poisoned muscle differ little if at all from those of unpoisoned muscle, indicating that in this instance the Meyerhof cycle can be temporarily replaced by other mechanisms.

The authors studied the effect of this substance upon an organism in which lactic acid formation is practically the whole metabolic story. *Streptobacterium casei* was chosen,[2, 3] a pure culture of which was kindly furnished by Dr. C. B. van Niel of the Hopkins Marine Station.

The bacteria were grown on agar plates, 0.2% glucose and containing $CaCO_3$. Mass cultures so prepared were scraped from the agar and suspended in sterile solutions of 0.2% glucose, 0.3% sodium bicarbonate. Lactic acid production was measured in the usual way, using Warburg respirometers in a thermostat at 21.5°C.[4] The sodiummonoiodoacetate was placed in the bulb or "pear" of the manometer vessel, and added to the suspension about an hour after the beginning of the experiment, so that each preparation furnished its own control. Blank determinations were also run, and proper corrections made.

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[1] Meyerhof, O., "Die Chemischen Vorgänge im Muskel". Berlin, 1930.

[2] Orla-Jensen, S., "The Lactic Acid Bacteria". Copenhagen, 1919.

[3] Kluyver, A. J., and Donker, H. J. L., *Proc. Roy. Acad. Amsterdam*, 1925, **28**, 605.

[4] Warburg, O., "Uber den Stoffwechsel der Tumoren". Berlin, 1926.

Briefly, it was found that concentrations of sodiummonoiodoacetate in the respirometer vessels of up to 0.008% had little effect upon the rate or extent of lactic acid production, while at a concentration of 0.08% a change in slope was noted 65 minutes after the addition of the reagent, and at a concentration of 0.16% such change in slope developed 35 minutes after addition. Both the rate and final amount of lactic acid formed decreased as the concentration of sodiummonoiodoacetate was raised. That this was not merely an osmotic effect is indicated by the fact that no such changes were observed in the presence of isomotic concentrations of sodium acetate.

The action of sodiummonoiodoacetate on the metabolism of the organisms with a substrate of methyl glyoxal, hexose phosphate esters, and other carbohydrate precursors of lactic acid is now being determined.

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Action of Sodiummonoiodoacetate on Aerobic and Anaerobic Glycolysis in Blood.

JOHN FIELD AND EDWIN S. PEEKE. (Introduced by E. G. Martin.)

From the Laboratory of Physiology, Stanford University.

The rates of the glycolytic processes in blood vary from animal to animal and class to class, being fairly rapid in man, dog and sheep, but slow in ox and pig.[1] The important enzymes are largely present in the formed elements of the blood.[2] Lundsgaard[3] has shown that glycolysis is inhibited by monoiodoacetate in concentrations which do not interfere with many other enzymic processes. It is well known that glycolysis is more rapid in nitrogen than in oxygen (Pasteur reaction), probably because the oxidative and fermentative enzyme systems are competing for a compound such as methyl glyoxal, important in each type of catabolism. Accordingly it seemed possible that the Lundsgaard reagent would be more effective in inhibiting aerobic than anaerobic glycolysis, since in the latter case there is but one anti-glycolytic mechanism, in the former in a sense there are two.

To test this assumption tubes were made up as follows, using blood from a single animal (dog) for each series of experiments: Defibrinated blood, 10.0 ml., 1% glucose 5.0 ml., Sorensen's phosphate buffer (pH 7.2) 10.0 ml., distilled water or various concentrations of sodium monoiodoacetate to volume of 40.0 ml.

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[1] Macleod, J. J. R., *J. Biol. Chem.*, 1913, **15**, 497.

[2] Katayama, L., *J. Lab. and Clin. Med.*, 1926, **12**, 239.

[3] Lundsgaard, E., *Biochem. Z.*, 1930, **217**, 162.

These tubes were placed in a thermostat at 37°C. for 4 to 5 hours. Oxygen-free nitrogen was passed through some of these, air through others, and the decrease in glucose then determined. With a concentration of Lundsgaard's reagent of 1.88 mg./ml. in the experimental tubes, the following data were obtained. A pair of experiments, a and b, were performed in each case.

	Loss of glucose in mg./ml. in 5 hours at 37°C.	
(1) Dog 14	a	b
Aerobic control	0.13	0.13
Anaerobic control	0.23	0.24
Aerobic-iodo acetate	0.00	0.00
Anaerobic-iodo acetate	0.17	0.17
(2) Dog 15		
Aerobic control	0.20	0.20
Anaerobic control	0.33	0.31
Aerobic-iodo acetate	0.00	0.00
Anaerobic-iodo acetate	0.23	0.24

With 1.4 mg. of iodo acetate per ml. in the experimental tubes.

(1) Dog 12		
Aerobic control	0.51	0.53
Anaerobic control	0.71	0.71
Aerobic-iodo acetate	0.18	0.18
Anaerobic-iodo acetate	0.27	0.26

	Loss of glucose in mg./ml.	
	a	b
(2) Dog 13		
Aerobic control	0.50	——
Anaerobic control	1.05	1.03
Aerobic-iodoacetate	0.12	0.10
Anaerobic-iodoacetate	0.20	0.22

The data indicate that anaerobiosis diminishes the inhibitory action of sodiummonoiodoacetate on glycolysis in dog's blood under the conditions of these experiments. The fate of the glucose disappearing, the rates of reaction, and the effect of arsenates and other agents modifying fermentative activity will be the subject of a subsequent communication.

5886

Monoiodoacetic Acid Poisoning in Albino Rats: Symptoms, Minimum Lethal Dose.

VICTOR E. HALL AND JOHN FIELD, 2ND. (Introduced by E. G. Martin.)

From the Laboratory of Physiology, Stanford University.

The finding of Lundsgaard[1] that monoiodoacetic acid injection into frogs inhibited the production of lactic acid by skeletal muscles on stimulation, without abolishing contraction, has revolutionized theories of the chemical mechanism of muscular contraction. Accordingly, it seemed valuable to prepare for the extension of this work to mammals by determination of the dosage required for the various symptoms, and for death.

Solutions of monoiodoacetic acid, brought to pH 7.2 by addition of sodium hydroxide, were injected intraperitoneally into unanesthetized female albino rats of 130 to 250 gm. weight, the dosage range being from 30 to 400 mg. per kg. body weight. A group of 5 animals received each dose. The rats had been starved for a period of 48 hours previous to injection.

In all animals receiving 120 or more mg. per kg. death occurred within 20 minutes. The time of onset of symptoms varied from 2 minutes with the largest dose to 8 minutes with 120 mg. per kg. The first alteration of behavior was the appearance of marked general hypoactivity, interrupted by sudden attacks of hyperactivity—running, climbing or biting, in which the movements soon became definitely ataxic. Circus movements appeared in a number of cases. Distinct hyperpnea next appeared, which often progressed to a definite inspiratory dyspnea. The hind legs became paralyzed, being dragged in an abducted posture. The animal soon became prostrate, lying on the side and making no righting movements if placed on the back. Occasionally tremors or mild clonic convulsions occurred. During various of the above stages the animals squeaked loudly, if touched or convulsed, in a manner suggesting hypersensitivity. Finally there appeared a violent tonic convulsion involving the whole body, lasting only about 10 seconds, but leaving the animal in extreme rigor in the posture given to it by the convulsion; mouth open, marked opisthotonus, elbows and wrists flexed; digits of fore feet flexed and adducted; hip, knee and ankle extended; digits of hind feet extended and abducted. The rigor was especially marked in the thoracic wall. Respiration ceased, with the occasional exception of some gasping movements in the head. The heart often continued to beat slowly for as much as a minute after the rigor had set in. The above course was presented by all the 25 animals, with the exception of 2, in which the terminal convulsions were weak and in which rigor did not appear until some minutes after death and was not extreme.

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[1] Lundsgaard, E., *Biochem. S.*, 1930, **217**, 162; **227**, 51.

All the animals receiving doses between 110 and 70 mg. per kg. died. A certain number (those dying within 30 minutes) presented the course described above for higher doses. However, with decreasing dose, an increasing number presented a much more prolonged survival, the earliest death in this second group being at 106 minutes after injection. Briefly their clinical course was: alternating periods of hypoactivity and hyperactivity with ataxia; prostration slowly developing, the animal losing righting responses and lying on the side; hyperpnea, passing into inspiratory dyspnea, each inspiration being accompanied in certain cases by general trunk and leg movements; hypersensitivity, passing into loss of sensitivity; attacks of clonic convulsions of variable duration and intensity, occasionally taking the form of rolling movements; fall of body temperature; slowing of respiration with development of irregularity; and finally death without terminal convulsions. Rigor never appeared before 15 minutes after death. All deaths occurring at times exceeding 100 minutes were preceded by the above course.

We believe that 2 fairly distinct clinical courses in the fatal poisoning may be distinguished: first, rapid sudden death, with terminal convulsions passing directly into rigor; second, slowly developing prostration with late death, rigor appearing after a definite interval.

All animals receiving 30 or 50 mg. per kg. survived at least 24 hours. These presented marked hypoactivity and moderate degrees of prostration, which reached its height in about 4 hours and per-

TABLE I.
Survival Times of Albino Rats Receiving Monoiodoacetic Acid.

Dose, mg. per kg.	Times of death min. after injection					50% Survival, Time
400	6	6	7	8	13	7.5
200	8	9	9	12	13	9.0
150	8	11	11	11	14	10.5
130	8	8	9	10	15	9.0
120	14	15	18	18	20	16.5
110	12	14	21	23	112	17.0
90	12	24	27	115	121	25.0
70	24	106	200	242	337	150.0
50	All over 24 hours					—
30	” ” ” ”					—

sisted unchanged for about 24 hours. No convulsions were observed.

In Table I there are tabulated for each dose, the time of death of each animal, and the time at which 50% of the animals remained alive. This last figure was obtained by plotting against time in minutes a curve representing the percentage of animals remaining alive at each minute; and then locating on the time axis the point where the smoothed curve crossed the 50% line. For doses of 90 mg. per kg. or greater, this value is determined solely by the animals presenting the first type clinical course; below 70 mg. per kg. solely by those presenting the second type.

The data indicate that the minimum dose killing any animal was 70 mg. per kg.; the maximum dose survived by any animal was 50 mg. per kg. The minimum lethal dose thus lies between 30 and 50 mg. per kg. Experimenters will be more interested in the doses producing the poisoning. The second type of clinical course (prolonged prostration, death without immediate rigor) was obtained in doses ranging from 70 to 110 mg. per kg., the minimum producing it in 50% of cases being 70 mg. per kg. The first type (convulsions with immedate rigor) was obtained with doses of 70 mg. and greater, the minimum producing death in this manner in 50% of cases being 90 mg. per kg.; and the minimum for 100% being 120 mg. per kg.

While this study has not included any analysis of the mechanisms involved, the impression is given that the earliest action on the intact animal is on the central nervous system, including particularly a stimulation of the respiratory center. Provided that the dose be high enough, and that the central nervous system action has produced vigorous convulsions, the rigor producing action on muscles, seen by Lundsgaard in frogs, is evident.

5887

Ultrasonic Radiation and Yeast Cells.

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A system capable of producing a series of high frequency waves in fluid may be constructed by placing a piezo-electric crystal of carefully prepared quartz in a di-electric liquid such as xylol. A pair of electrodes is then placed upon opposite sides of the quartz crystal and should be connected with a high frequency oscillator. Such an arrangement of apparatus allows one to set up compression waves even of radio frequency in the liquid. A system similar to this has been described by Wood and Loomis.[1] Radiation by the resultant ultrasonic vibration has been shown by Wood and Loomis[1] to be destructive to certain varieties of organisms including algae, but it was their opinion that bacteria are not injured by such treatment. Radiation by ultrasonic vibration may be attended by cavitation of included gases and it is considered probable by Johnson[2] and by Schmitt and Uhlemeyer[3] that this mechanical expulsion of the gases is the cause of disruption of living cells. Recent observations of Wu and Liu[4] indicate that these rays coagulate protein and thus it becomes probable that protein changes contribute to the cause of death.

To test the effects of ultrasonic radiation upon yeasts, the following series of experiments was performed: A wine yeast was incubated in grape juice for 24 to 48 hours at 37°C. This organism, listed in our cultures as a *Saccharomyces elliposoideus,* was chosen because of its relatively large size and active growth.

Treatment was carried out by placing the culture to a depth of approximately one inch within a glass tube 1½ inches in diameter. The bottom of this tube, however, was of heavy cellophane cemented to the side walls. The culture within this tube was now lowered into the di-electric of xylol in which had been placed the piezo crystal of quartz. Thus the yeast culture was separated from the xylol only by a cellophane layer in order that the minimum amount of energy absorption might take place during transmission through the intervening wall. When current was applied a standing wave system appeared attended by formation of a pyramid of fluid in violent agitation and by much fog of finely divided fluid. Temperature tended to rise but a circulatory cooling system held the thermometer reading below 39°C. At least 25 tests of the effect of ultrasonic vibration upon this and other similar yeasts have been made with results indicated in Table I. Figures are to be translated not as counts per cc. but rather as indicators of the relative numbers of viable forms present. They were obtained by plating upon Sabouraud's agar.

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[1] Wood, R. W., and Loomis, A. L., *London, Edinburgh and Dublin Philos. Mag. and J. of Sci.*, 1927, **4**, 417.

[2] Johnson, C. H., *J. Physiol.*, 1929, **67**, 356.

[3] Schmitt, F. O., and Uhlenmeyer, B., Proc. Soc. Exp. Biol. and Med., 1929, **27**, 626.

[4] Wu, H., and Liu, S., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 782.

TABLE I.
The Effectiveness of Ultrasonic Vibration in Killing Actively Growing Yeasts.

Before Treatment	100,000	28,000	54,000	50,000	21,000	22,800	15,000	125,000	36,500
1 min.	17,000	39,000	2,100	1,800	12,600	22,800	12,500	31,500	4,200
3 ”	0	4,300	0	450	9,100	9,100	10,500	3,000	8
5 ”	0	110	0	20	3,500	7,700	11,200	360	5
10 ”	0	21	0	8	2,200	1,750	5,800	13	5
15 ”	0	0	0	0	1,600	lost	3,500	1	5

Microscopic examination of treated culture material revealed much cellular debris and relatively few formed yeast cells. Moreover, transfer from a treated culture to a fresh tube of medium in many instances was followed by no subsequent growth, indicating, therefore, complete sterilization. In other instances, however, these subcultures did show delayed growth.

It appears, therefore, that ultrasonic radiation is an effective agent in killing yeasts. Moreover, the death of the yeast cell may be attended by its disruption.

5888

Prevention of Heat-Coagulation, Mercuric-Precipitation of Proteins, and of Precipitation of Alkaloids by Colloidal Dyes.

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Congo-red and some other colloidal dyes possess the unique action of preventing death in animals injected with surely fatal doses of potent bacterial toxins and drugs.[1] This protective action appears to be mediated through the physical properties of these colloidal dyes. Presumably correlated with this physical protection *in vivo* are some striking protective effects of the colloidal dyes on powerful reactions *in vitro,* such as the heat-coagulation and mercuric precipitation of proteins and the alkaloidal precipitation by Mayer's reagent. As far as I know, these marked protective effects *in vitro* have not been previously described, but they may have considerable scientific importance and practical usefulness.

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[1] Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.

Heat-coagulation of proteins. Using equal parts of the dyes in 1% strength in 6% dextrose solution, or in distilled water, and of whole serum or saturated solution of dry egg albumin, the following dyes prevented coagulation of the mixtures when boiled: congo red, vital red, trypan red, mercurochrome, rose bengal and sodium fluorescein. Congo red also prevented the heat-coagulation of 50% raw egg white in salt solution. Coagulation did not occur after continuous (half-hour) boiling, and then cooling, of the dye-protein mixtures. The control mixtures, without dyes, were coagulated in less than 1 minute, and after cooling, frequently solidified. The minimum protective amount of congo red for 1 cc. of serum was about 2.5 mg., and certainly protective, was 5 mg. The other dyes were not quantitated, but appeared to be about as efficient as congo red. Acriflavine, which is colloidal according to Hirschfelder and Wright,[2] was protective, but acted somewhat peculiarly: weak and strong solutions precipitated serum, but only the precipitate of strong solutions redissolved in small excess of the solution and such mixtures did not coagulate on boiling. Salyrgan was effective in preventing heat-coagulation of serum. Partially or doubtfully protective were azo blue and trypan blue.

The following colloidal dyes did not prevent heat-coagulation of serum and egg albumin under the same conditions: toluidine blue, neutral red, diamine blue, alkali blue, Chicago blue, aniline blue, induline, nigrosine, new fast green B, night blue, and benzopurpurin. Such colloids as acacia, gelatin, soluble agar, arsenious sulphide, iron, protargentum and indigocarmine did not prevent coagulation of serum. The following crystalloidal dyes did not prevent coagulation of serum and egg albumin; methylene blue, methyl violet, methyl red, phenol red, basic fuchsin, leucosulphato green, sulphato violet, sulphato green, crystal violet (gentian violet).

In all cases, the boiled and protected dye serum mixtures immediately precipitated after the addition of weak solutions of calcium chloride and magnesium sulphate and of saturated sodium chloride, but not of 0.9% sodium chloride and of strong sodium iodide. Under these conditions, the dye was removed from solution with the flocculent precipitate. The salt-effects, but especially those of the weak chloride and the strong iodide, suggest that the physical factor in the protective action of the dyes on heat-coagulation of the native proteins is not due to interference with the action of the natural salt (adsorption by the dye), which is essential to heat-coagulation. Rather it would seem that the protective mechanism is in the nature of a surface (peptizing?) action of the dyes on the protein aggregates. In support of this action was the observation that anything which destroyed the physical properties (surface activity) of the dyes impaired or destroyed their protective action, such as the use of a dye in salt solution, the addition of a polyvalent ion or of acid to the dye (congo red), or to the boiled dye-protein mixture, or the addition of mercuric chloride to the latter when hot. Bile salts and caprylic alcohol, which lower surface tension, did not affect the protective action of congo red.

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[2] Hirschfelder and Wright, *J. Pharm. Exp. Therap.*, 1930, **39**, 13, 411 and 433.

Mercuric chloride precipitation. In the cold, the precipitation of 50% horse-serum and of saturated solution of dry egg-albumin by mercuric chloride, using about 0.05 to 0.1 cc. of 1% mercuric chloride solution to 1 cc. of dye-protein mixture, was prevented by the same dyes which were effective in preventing the heat-coagulation of proteins, while the controls without dyes precipitated immediately. A large excess of mercuric chloride caused some precipitation. It was interesting to observe that the organic mercurial, mercurochrome (2:7 dibromo-4-hydroxy-mercurifluorescein), when first added to serum or egg-albumin, was very effective in preventing the precipitating action of the inorganic compound, mercuric chloride. This is attributed to the colloidal nature of the mercurochrome, recently demonstrated by Hirschfelder and Wright[2] in their studies on the colloid chemistry of antisepsis. In support of the fact that the protective action was associated with the dye-complex of the mercurochrome and not with the mercury ion were the positive protective effects of rose bengal (tetraiododichlorofluorescein) and of sodium fluorescein on the precipitating action of mercuric chloride. As indicated by the results of gelatin-diffusion experiments, it appeared that rose bengal was more colloidal than mercurochrome, and consistent with this property was a high protective efficiency of rose bengal on the heat-coagulation and mercuric-precipitation of the proteins.

Precipitation of alkaloids. Saturated or strong solutions in water of strychnine, morphine, codeine, papaverine, cocaine, atropine, quinine, quinidine, nicotine and curarine, mixed with equal parts of 1% congo red in 6% dextrose solution, did not precipitate after the addition of potassium mercuric iodide (Mayer's reagent). The addition of the dye after preliminary precipitation of the alkaloids left the mixtures turbid or precipitated. Other dyes which prevented precipitation of a saturated solution of strychnine by Mayer's reagent were: vital red, rose bengal and mercurochrome. The addition of calcium chloride solution to these strychnine-dye mixtures caused immediate precipitation. Trypan blue, trypan red and azo blue did not protect or were indefinite against alkaloidal precipitation. In general, the colloidal dyes which were effective in preventing heat-coagulation and mercuric-precipitation of proteins were also effective in preventing alkaloidal precipitation.

Comment. The full significance of the phenomena here described is not yet apparent, but a few suggestions may be in order. A common physical mechanism for the various protective effects *in vitro* exerted by the colloidal dyes, chemically different, appears to be the basis of these striking phenomena, which suggests that a similar physical mechanism is responsible for their protective effects against the fatal actions of drugs and toxins *in vivo.* Dyes with crystalloidal properties are obviously ineffective, which is also the case with a number of electronegative colloidal dyes and agents tried, possibly because the latter do not consist of sufficiently small aggregates or micellae. Electropositive colloids obviously would not protect. Some of the protective agents here described reputedly act as systemic antiseptics. This encourages the belief that the effects exerted in virtue of the colloidal properties of chemotherapeutic agents may be important in their therapeutic actions. The variety of physically and chemically different agents, useful in the treatment of certain parasitic and infectious diseases, justifies the thought that some common property actuates or supports their therapeutic efficiency. Consider, for example, the variety of agents which are effective in the treatment of syphilis: arsphenamines, mercury, bismuth, tellurium, vanadium, iodide, pyrexia (malaria, sulphur, vaccine), tryparsamide, etc. The mediation of therapeutic effects through the tissues would not be eliminated from the consideration of the colloidal and physico-chemical factors, but should be given greater weight, since it is probable, in fact known to some extent, that crystalloidal chemotherapeutic agents acquire the properties of colloids under biological conditions, and that other agencies affect the tissues and their fluids in the sense of colloidal systems. The implied principle of general protection, through colloidal properties, against the injuries of the virus and products of disease, thus promoting natural defenses, would lack the specific connotations of chemical groups or ions, but could conceivably assist the latter, have broader applications, and explain the results in unrelated conditions.

Conclusions. Certain colloidal dyes, namely, congo red, vital red, trypan red, mercurochrome, rose bengal and sodium fluorescein, strikingly prevent the heat-coagulation and mercuric-precipitation of horse serum and egg albumin, and also the alkaloidal precipitation by Mayer's reagent. These powerful protective actions *in vitro* appear to be associated with the physical (colloidal) properties of these dyes, in agreement with a similar mechanism of protection exerted by some of these dyes on the actions of potent toxins and drugs *in vivo*. The possible significance of the mechanism of these common protective phenomena, for chemotherapy in general, is briefly discussed.

5889

The Toxicity of Nupercaine.*

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Among the requirements of an ideal local anesthetic agent is prolongation of action. Attempts have been made to meet this requirement, especially in connection with quinine derivatives, but tissue injury at the site of injection has generally precluded clinical use of such agents.[1] Uhlmann[2] introduced the diethyl-ethylene-diamide of butyloxycinchoninic acid (nupercaine, N.N.R.). In Europe this drug came into immediate favor, but a number of deaths were reported[3] following its use. Papers on the toxicity of nupercaine have been published,[4] but since these are not in agreement and did not take up the question of protection, further study seemed indicated.

We began with a 1% solution of nupercaine, but due to tissue irritation, we changed to a 0.5% solution. Rabbits were used and the drug was given subcutaneously or intravenously, with or without

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* Supported in part by a grant from the American Dental Association.

† Fellow of the National Research Council.

[1] Dixon, W. E., and Premankur De, *J. Pharmacol. Exp. Therap.*, 1927, **31**, 407. Millzner, R. J., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 526.

[2] Uhlmann, F., *Narkose u. Anesth.*, 1929, **2**, 168; *Arch. Internat. Pharmaco. Therap.*, 1929, **36**, 253.

[3] Keyes, E. L., and McLellan, A. M., *Am. J. Surg.*, 1930, **9**, 1.

[4] Bond, W. R., and Bloom, N., *J. Lab. Clin. Med.*, 1931, **16**, 447. Laubender, W., *Deutsche Med. Wchnschr.*, 1930, **56**, 1658. Lipshitz, W., and Laubender, W., *Klin. Wchnschr.*, 1930, **9**, 968.

protection. For protection we used paraldehyde administered in water emulsion by stomach tube.

Subcutaneous injection of nupercaine gave rise to convulsions on an average of 17 minutes from the time of injection. During this period there was first depression, then followed nervous excitement with loss of coordination, and finally clonic convulsions. Respiration became rapid, while the pulse was slow and irregular. Those animals recovering took 2 to 3 hours before they seemed normal. In those that died, the respiration stopped a few seconds before the pulse. The lethal dose (LD_{50}) of 8.5 mg./kg. is one-twelfth of the minimal lethal dose for cocaine given subcutaneously as reported by Knoefel, Herwick, and Loevenhart.[5]

Rabbits receiving an intravenous lethal dose of nupercaine died in less than a minute, and usually without any muscular effect whatever, although some had one severe convulsion. Kymographic studies indicated that nupercaine is similar in action to cocaine on intravenous administration in lethal doses.[5] Those receiving a tolerated dose showed the same symptoms as those given subcutaneous injections, but the reaction set in immediately and did not last as long. The lethal dose (LD_{50}) of 2.5 mg./kg. is one-sixth of the minimal lethal dose for cocaine given intravenously.

The rabbits receiving paraldehyde by stomach tube before the subcutaneous injection of nupercaine show the amount of protection offered by a typical cerebral depressant. In animals under the influence of paraldehyde, very little reaction to nupercaine is noted. A slight twitching of the legs and a marked slowing of the pulse is

TABLE I.
Data on Toxicity of Nupercaine in Rabbits.

Mode of Administration	Dosage in mg./kg.	Animals Used	No. Died	No. Lived
Injected subcutaneously	5	8	0	8
	7.5	8	3	5
	10	8	5	3
	15	5	5	0
” intravenously	2	8	2	6
	3	8	6	2
	5	5	5	0
” subcutaneously with 15 mm./kg. paraldehyde orally at same time	10	8	1	7
	20	8	2	6
	30	4	4	0
Injected intravenously when 15 mm./kg. paraldehyde had been given orally 15 minutes before	5	8	6	2

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[5] Knoefel, P. K., Herwick, R. P., and Loevenhart, A. S., *J. Pharmacol. Exp. Therap.*, 1930, **29**, 397.

usually observed. As in the case of other types of local anesthetics, hypnotics protect against nupercaine injected subcutaneously, increasing the lethal dose about 3 times, but on intravenous injection, little protection is obtained.

Absorption of nupercaine when given subcutaneously is very slow, but it is 12 times more toxic than cocaine when given in that manner, and only 6 times more toxic if given intravenously. This indicates that, compared with cocaine, nupercaine attacks the central nervous system more readily than the heart. All the phenomena observed in this study are in accord with that explanation of intoxication from local anesthetics which has already been discussed.[5]

5890

Preservatives for Bacteriophage Suspensions.

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That bacteriophage possesses therapeutic value in certain types of bacterial infections is becoming more and more evident.[1] The increasing demand for 'phages intended for therapeutic use raises a question, applicable to all biological products, as to the choice of a suitable preservative. While filtration through a sound candle generally fulfills the requirements as to sterility, it is well known that this is not absolute proof against the development of so-called "secondary cultures". Besides this there is also the possibility of accidentally introducing organisms in ampouling the filtrate for shipment.

In choosing preservatives for 'phage products 2 considerations must be borne in mind: (1) The preservative must be bactericidal without in any way impairing the potency of the 'phage and (2) the concentration of the germicide which is employed should be such that it will not be toxic for the patient in the amounts which would be administered with large doses of 'phage. In studies designed to elicit the value of 'phage as a therapeutic agent, a third consideration enters. Here it is important to reduce the concentration to a level which will insure bacteriostasis in the ampoule and will not, on further dilution by body fluids (urine, etc.) after administration of the 'phage, exercise an appreciable bactericidal effect *in vivo.*

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[1] Schultz, E. W., *California and Western Medicine*, 1932, in press.

Of a number of substances tested merthiolate,* metaphen, and potassium mercuric iodide seemed essentially to fulfill the above requirements. Jamieson and Powell[2] have reported that merthiolate in a concentration of 1-5,000 has little effect on the activity of an antistaphylococcus bacteriophage at room or ice box temperature. They also reported that "bacteriophage containing as much as 1% merthiolate for several weeks produces high titered lysing of fresh cultures."

In the studies reported below a freshly prepared solution of merthiolate prevented growth in a dilution of the powdered reagent of 1-1,000,000 in heavily inoculated broth cultures of *Esch. coli* and *S. aureus.* The bacteriostatic activity of this solution of merthiolate was reduced to 1-100,000 after standing several weeks. Metaphen prevented growth in a dilution of 1-100,000 and potassium mercuric iodide, 1-10,000.

Results obtained with various concentrations of the above agents

TABLE I.

'Phage	Days Incubation	Visible Lysis in Dilution of			
		Merthiolate 1-10,000	Merthiolate 1-100,000	Metaphen 1-100,000	K_2HgI_4 1-10,000
Antistaph. No. 15 AD-52	0	10^{-9}	10^{-9}	10^{-9}	10^{-10}
	5	10^{-6}	10^{-9}	10^{-8}	10^{-8}
	10	10^{-3}	10^{-10}	10^{-9}	10^{-9}
	20	10^{-4}	10^{-9}	10^{-9}	10^{-9}
	30	10^{-2}	10^{-8}	10^{-9}	10^{-9}
Anticoli No. 2 K-13	0	10^{-8}	10^{-8}	10^{-8}	10^{-8}
	5	10^{-7}	10^{-8}	10^{-7}	10^{-8}
	10	10^{-7}	10^{-8}	10^{-8}	10^{-7}
	20	10^{-6}	10^{-8}	10^{-8}	10^{-8}
	30	10^{-5}	10^{-8}	10^{-7}	10^{-7}
Anticoli No. 77 K-199	0	10^{-6}	10^{-6}	10^{-6}	10^{-6}
	5	10^{-5}	10^{-7}	10^{-5}	10^{-5}
	10	10^{-6}	10^{-5}	10^{-5}	10^{-4}
	20	10^{-4}	10^{-6}	10^{-6}	10^{-5}
	30	10^{-3}	10^{-6}	10^{-5}	10^{-5}
Anticoli No. 98 K-222	0	10^{-5}	10^{-5}	10^{-5}	10^{-5}
	5	10^{-4}	10^{-5}	10^{-5}	10^{-4}
	10	10^{-4}	10^{-4}	10^{-4}	10^{-5}
	20	10^{-3}	10^{-4}	10^{-4}	10^{-4}
	30	0	10^{-4}	10^{-4}	10^{-4}

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* I am indebted to Dr. W. A. Jamieson of Eli Lilly and Company for supplying me with the merthiolate used in this work.

[2] Jamieson, W. A., and Powell, H. M., *Am. J. Hyg.*, 1931, **14**, 218.

in antistaphylococcus and anticoli bacteriophages incubated for one month at 37.5°C. are reported in Table I, the activity of the 'phage suspension being determined by periodic titration during this period. No secondary cultures developed in any of the chemically treated suspensions while secondary cultures developed in several of the filtered 'phage controls.

These results indicate that merthiolate can be added in a concentration of 1-100,000, metaphen 1-100,000, or potassium mercuric iodide 1-10,000 to suspensions of 'phages active against *Esch. coli* or *Staph. aureus* without reducing the titer of the 'phage on standing and at the same time preventing the development of secondary cultures.

At 37.5°C. the results reported here with merthiolate do not agree with those reported by Jamieson and Powell for tests carried out at a lower temperature. Results given in Table I show that the activity of the various bacteriophages tested was slowly reduced by merthiolate in a concentration of 1-10,000. Jamieson and Powell reported that merthiolate in a dilution of 1-5,000 did not markedly affect the 'phage in one year at room or ice box temperature. In these tests at 37.5°C. merthiolate in a concentration of 1-1,000 completely inactivated an antistaphylococcus bacteriophage in less than 2 weeks.

By the use of such preservatives it should, therefore, be possible for the physician located at a distance from the bacteriophage supply laboratory to maintain an efficient supply of various polyvalent 'phages available for use in emergency or routine therapeutic use of bacteriophage.

5891

A Method for the Collection of Blood from Rats.

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Various routes have been used for obtaining blood from rats. Some of these are: the heart, femoral vein, and the tail. All these routes have been found unsatisfactory for our work. The heart puncture is too dangerous since the number of accidents and deaths is very large. The tail was found unsatisfactory since the amount of blood one could get was small. It also exposes the animal to infection. The femoral vein is very small in rats. We were not able to locate it without cutting the skin. The jugular vein is by far the largest of the peripheral vessels of the rat. Blood may be drawn from it without much difficulty because of the ease in locating it by its pronounced pulsation. The jugular vein of the rat runs anteriorly *over* the clavicle about 10 mm. from the middle of the sternum.

If a rat is given a little ether, placed on its back on a board and tied so that the head is approximately one inch lower than the rest of the body, the hair clipped over the clavicle, it is easy to locate the jugular by the pulsation and to feel the clavicle. With the clavicle to support the vessel and the pulsation to direct us, it is not difficult to place the needle into the vessel. This same route has been used by us for injecting various substances and also for transfusing rats.

It has also been found possible to collect blood from rats according to the above technique without the use of an anesthetic.

5892

Studies on Calcium and Phosphorus in Bile-fistula Dogs.

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The work of Pavlov,[1] Looser,[2] Wisner and Whipple,[3] Düttman,[4] Dietrich,[5] Gilbert,[6] and Buchbinder and Kern[7] has shown that absence of bile from the intestinal tract of animals leads to osteoporosis. The gross manifestations of the condition are softening of the bones and spontaneous fracture. The condition is not brought about when only small amounts of bile are permitted to enter the intestinal tract. Results in accord with the above were obtained by Seidel[8] in 2 human cases. Two explanations to account for this phenomenon have been advanced: (a) Klinke[9] postulates that in the absence of bile there is lack of calcium absorption, (b) Seifert[10] suggests that in the absence of bile, vitamin D is not absorbed. Support for the latter hypothesis is furnished by Tammann[11] who showed that parenteral administration of vitamin D alleviated the condition.

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[1] Pavlov, I. P., *Verein. Ges. russ. Aertzte*, 1904, **72**, 314.

[2] Looser, E., *Verhandl. deut. Path. Ges.*, 1907, **11**, 291.

[3] Wisner, F. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, **60**, 119.

[4] Düttmann, G., *Beitr. klin. Chir.*, 1923, **139**, 720.

[5] Dietrich, H., *Beitr. klin. Chir.*, 1925, **134**, 530.

[6] Gilbert, E., *Z. Ges. exp. Med.*, 1924, **43**, 539.

[7] Buchbinder, W. C., and Kern, R., *Arch. Int. Med.*, 1927, **40**, 900; *Am. J. Physiol.*, 1927, **80**, 273.

[8] Seidel, H., *Münch med. Wochschr.*, 1910, **57**, 2034.

[9] Klinke, K., *Klin. Wochschr.*, 1928, **1**, 385; *Ergeb. d. Physiol.*, 1928, **26**, 279.

There has been no systematic work dealing with calcium and phosphorus balances in bile fistula dogs. King, Bigelow and Pearce[12] reported negative calcium balances over a period of a week and Emerson[13] reported decreased fecal calcium output over several 3-day periods. However, from the standpoint of a calcium balance Emerson's results have little significance.

In the present work, calcium and phosphorus balances were carried out on 2 female dogs in which bile was excluded from the intestinal tract by cholecystonephrostomy. This operation was

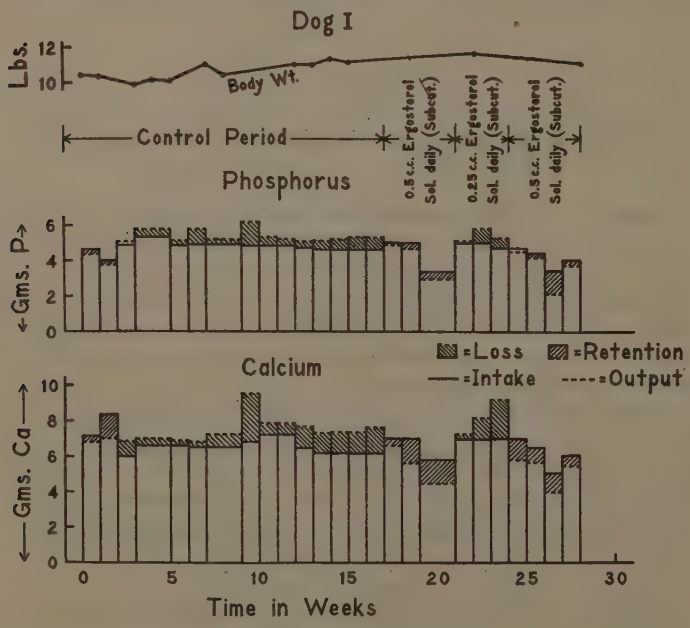

FIG. 1.

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[10] Seifert, E., *Beitr. klin. Chir.*, 1926, **136**, 496.

[11] Tammann, H., *Beitr. klin. Chir.*, 1928, **142**, 83.

[12] King, J. H., Bigelow, J. E., and Pearce, L., *J. Exp. Med.*, 1911, **14**, 159.

[13] Emerson, W. C., *J. Lab. Clin. Med.*, 1928, **14**, 122.

kindly performed for us by Dr. C. M. Johnson according to the technique described by Kapsinow, Engle and Harvey.[14] These animals were fed a miscellaneous diet, comparatively low in fat, for periods of 128 and 189 days respectively, after which they were placed on a diet consisting of casein 25 parts, cracker meal 48 parts, sucrose 5 parts, vitavose 18 parts, agar 2 parts, and salt mixture (calcium carbonate 1 part, sodium chloride 2 parts, magnesium carbonate 0.2 part) 2 parts. An adjustment period of one to 2 weeks was allowed before calcium and phosphorus balances were begun. For dog II, urine and feces were analyzed separately, while for dog I, composite analyses only were made.

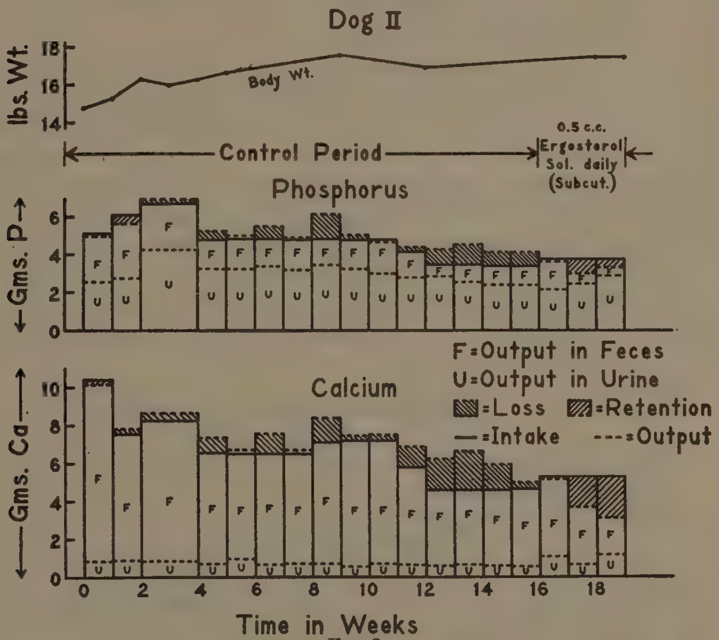

FIG. 2.

The balance data are presented in Figs. 1 and 2. It will be noted that dog I was in negative calcium and phosphorus balance during a period of 17 weeks. On administration of 0.5 cc. Viosterol (Squibb 100 D) solution daily for a period of 4 weeks both the calcium and phosphorus balances became positive. When this dose was decreased to one-half of this amount, both the calcium and the phosphorus balances became negative. On increasing the dose of viosterol solution to 0.5 cc., both the calcium and the phosphorus balances again became positive. Similar results were obtained with dog II. Administration of 0.5 cc. solution of viosterol subcutaneously shifted a 16 weeks' negative calcium and phosphorus balance to a positive balance.

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[14] Kapsinow, R., Engle, L. P., and Harvey, S. C., *Surg. Gynec. and Obs.*, 1924, **39**, 62.

We are also reporting some data dealing with the distribution of blood calcium in these dogs. Sekitoo[15] has reported low blood calcium in bile fistula rabbits. Emerson[13] found a gradual rise in diffusible and blood calcium in his internal bile fistula dogs over an 84-day period. However, the low values for diffusible calcium which Emerson reports without apparently any indications of tetany cast considerable doubt on his results.

Our results are given graphically in Fig. 3. In both dogs during the periods of negative calcium balance the diffusible calcium was higher, while during the period of positive calcium balance, the values reached a lower and more nearly normal value. During the periods of negative phosphorus balance the serum phosphorus in both animals was low. This value was increased to a more nearly normal value when the animal was in positive phosphorus balance.

It was necessary to terminate the experiments for uncontrollable reasons. The results while incomplete nevertheless point strongly to a depletion of vitamin D in the bile fistula dogs as the possible cause

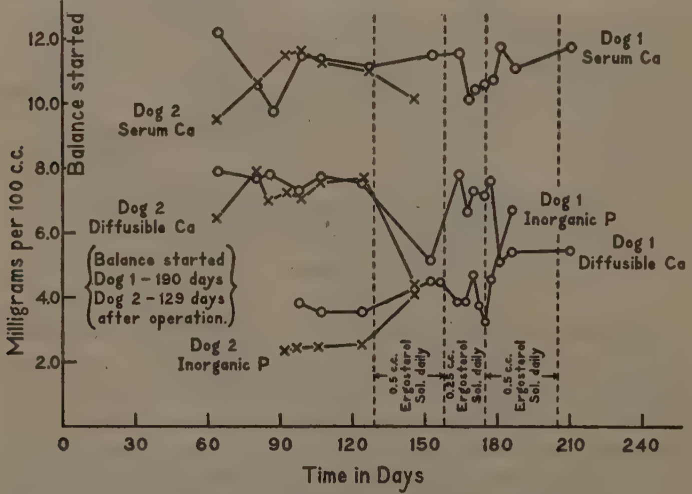

FIG. 3.

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[15] Sekitoo, T., *J. Biochem.* (Tokio), 1930, **11**, 391.

for the osteoporosis. It is possible that in normal animals, deoxycholic acid acts as a carrier for the absorption of vitamin D across the intestines. If so, it follows that in the absence of bile, little or no absorption of vitamin D would take place.[16]

It is hoped to resume experimental work in this field at an early date.

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[16] Schmidt, W., and Schmidt, C. L. A., *Univ. Calif. Pub. Physiol.*, 1930, **17**, 211.

Illinois Section.

University of Illinois, December 8, 1931.

5893

Effect of Replacement Therapy on Eruption of the Incisor of the Hypophysectomized Rat.*

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A series of 7 hypophysectomized rats and their litter mate controls was treated for various periods with daily injections (usually 20) of the growth-promoting hormone of the pituitary body. The experimental animals were operated upon at the age of 45 to 64 days. The rate of eruption of the incisors of these animals was measured for varying lengths of time preceding, during, and after replacement therapy (Table I).

A horizontal marking with a fine file (Swiss jeweler's screw head file) was made near the gingival line along the disto-labial margin of the upper incisor. With an adjustable caliper provided with fine points the distance was measured between the point where the marking crossed the disto-labial margin and the point where the surface of the gingivae crossed the same margin. This distance was read in twentieths of a millimeter with the aid of a stage micrometer and a dissecting microscope. The distance between the same points mentioned above was read 7 days later. The difference between the 2 readings represented the rate of eruption for that period. New marks had to be made each week in the normally erupting teeth. Operated and control animals received the same preparation† subcutaneously once daily in a dose proportional to the body-weight.

To obtain some idea of the accuracy of the individual measure-

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* This work was in part supported by the John D. Hertz Fund of the University of Chicago.

† Made by the method of van Dyke and Wallen-Lawrence and kindly furnished by the Wilson Laboratories.

ments, 8 experiments on teeth of different lengths were performed. Each experiment was made up of 4 measurements on the same tooth. The standard deviations calculated for these experiments were found to have the following values expressed as percentages of the mean values: 1.80, 5.26, 8.44, 7.94, 5.65, 6.44, 11.20, and 4.48. These deviations are probably greater than the actual ones because these experiments were performed at the beginning of this study and the technique has improved since then.

TABLE I.

Effect of Growth-Promoting Hormone on Rate of Eruption of Upper Incisors of Hypophysectomized Rats.

No.	Period of Injection		Maximum Wt. Gain		Approximate Average Weekly Rate of Eruption in mm.					% of Increase
	No. of Successive Days	Days Following Hypophysectomy	gm.	%	3 weeks preceding therapy	During therapy Mean	During therapy Max. per wk.	1st week after therapy	1st 3 wks after therapy	
314	20	54-73	68	60.7	0.18	0.95	1.3	0.75	0.38	427
324	20	77-96	46	40.4	0.36	0.8	1.2			122
319	20	89-108	0	0	0	0	0			0
268	21	125-145	28	21.7	0.33	0.66	0.9	0.95	0.5	100
317	12	139-150	32	22.4	0.10	0.26	0.45	0.24	0.14*	160
268	20	167-186	15	10.5	0.27†	0.63	0.75	0.25	0.25	137
268	20	224-243	0	0	0.20	0.1	0.2			0
258	21	294-314	43	27.4	0.29	0.51	0.75	0.2	0.25	75
258	20	336-355	39	22.3	0.27†	0.45	0.75	0.85	0.61	66
258	20	393-412	0	0	0.56	0.38	0.55			0
186	17	412-428	0	0	0	0				0

*First 17 days after replacement therapy.

†This average is based on the records of only 2 weeks preceding the treatment because the rate of eruption of the third week was still increased by a previous period of replacement therapy which was administered 4 weeks before.

The degree of retardation in the eruption-rate of the rat incisor

TABLE II.

Comparison of Mean Rates of Eruption of Upper Incisors of Normal and Hypophysectomized Rats.

Group	Number of Individuals	Number of Measurements	Mean weekly Eruption rate mm.	Standard Deviation mm.
I Control	14	190	1.980	±0.377
II Treated Control	10*	49	2.028	±0.838
III Hypophysectomized	5†	20	0.210	±0.163
IV Treated Hypophysectomized	5†	26	0.610	±0.324

*From Group I.
†Same individuals.

after hypophysectomy is summarized by the data of Table II. In the computation of the data, those obtained from 2 of the hypophysectomized rats are not included since the incisors of these animals were no longer erupting. There was unquestionably a significant difference between the eruption rates of the teeth of Groups I and III. The mean rate of incisor-eruption was not significantly altered in the normal animal by the administration of growth-promoting hormone although the body-weight was definitely increased (Groups I and II).

The rate of eruption of the rat incisors which is retarded following hypophysectomy[1] can be accelerated by replacement therapy. The increase in the rate of eruption can be observed within a week following the beginning of replacement therapy and continues for about a week after treatment is stopped. Except for some individual variations, the acceleration is greatest in the animals operated upon most recently and appears to become smaller as the interval between the time of the operation and the time of replacement therapy increases (Table I, Fig. 1). Two animals that were given replacement therapy during the fourteenth month following hypophysectomy (Rats 186, 258) showed no change in the rate of eruption of their incisors. Another animal (Rat 268) responded to replacement therapy when administered during the fifth and sixth month following the operation but showed no response to treatment during the eighth month following hypophysectomy (Fig. 1). The fact that neither control nor hypophysectomized animal responded to the third series of injections was not due to an inactive hormone-preparation; other animals, normal and operated, responded in a typical fashion from the same preparation administered

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[1] Schour, I., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 934.

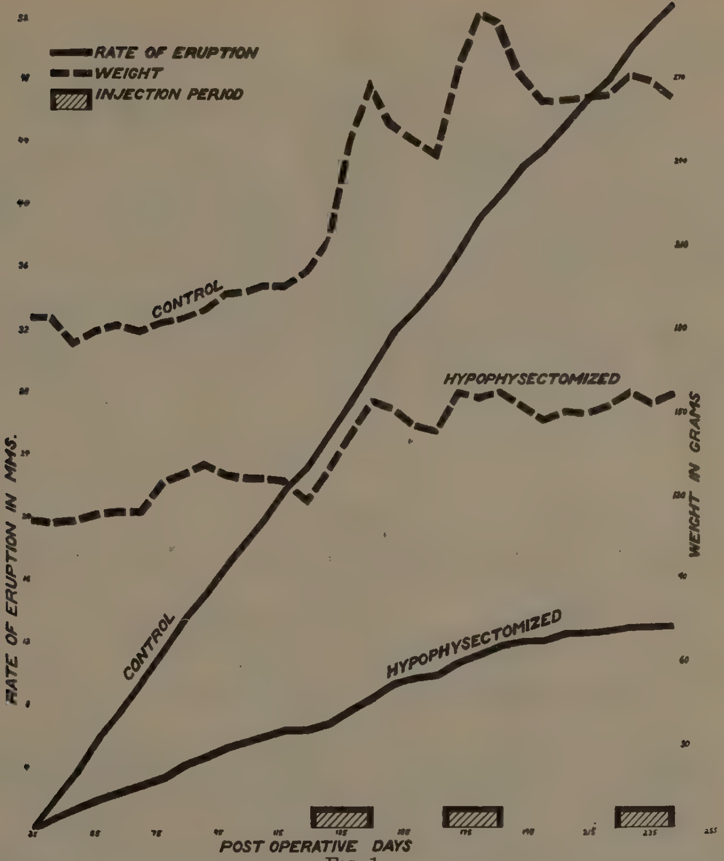

FIG. 1.

Effect of treatment on weight and incisor-eruption rate of hypophysectomized rat (268) and litter-mate control (266).

at the same time. One animal (Rat 319) did not respond during the fourth month following hypophysectomy. The atypical reaction of this animal may be associated with the fact that it was ovariectomized previous to the time of replacement therapy. However, in all the hypophysectomized animals, increase in weight and acceleration in eruption-rate were always associated.

The data in Table II on 5 of the hypophysectomized rats were selected as follows: the last 2 weekly measurements before treatment are the source of the data for Group III; the measurements during treatment as well as in the first week after treatment are the source of the data for Group IV. If the mean eruption-rates of these 2 groups are compared by Fisher's method,[2] they are found to be significantly different ($t = 4.94$).

Summary. 1. Following hypophysectomy the eruption of the incisor of the rat becomes progressively slower and finally ceases.

2. If not administered too late after operation, growth-promoting hormone increases the eruption-rate of the incisor. An increase in body-weight, following the administration of hormone to the hypophysectomized animal, is always associated with an increase in the rate of the eruption of the incisor.

3. In the normal animal, the administration of growth-promoting hormone under the experimental conditions described, although definitely increasing body-weight, does not appreciably alter the eruption-rate of the incisor.

5894

Production of Ulcers in the Prostomach of Rats by Protein Restriction.

FREDERICK HOELZEL AND ESTHER DA COSTA.
(Introduced by A. J. Carlson.)
From the Department of Physiology, University of Chicago.

Büchner, Siebert and Molloy[1] found that marked ulceration could be produced in the prostomach of rats by stimulating the gastric secretion with histamine on starvation days. Some ulceration also occurred in rats starved every other day during a period of 17 days, without histamine. Bürkle-de la Camp[2] confirmed these results with histamine but did not observe ulcers in the prostomach of rats after short periods of simple starvation. The question of ulceration following starvation was of particular interest because irritation of the parapyloric region by the gastric secretion was believed to explain a peculiar and intense hunger sensation that repeatedly developed with starvation or protein restriction in the senior author of the present report (Hoelzel and Kleitman[3]). Moreover, the observation that protein restriction led to an increase in the fasting gastric acidity (Hoelzel[4]) made it appear that, if ulcers would develop in the prostomach of rats with starvation, ulceration might also occur as a result of protein restriction alone.

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[2] Fisher, R. A., *Statistical Methods for Research Workers*, 1930, 107.

[1] Büchner, Siebert and Molloy, *Beiträge z. path. Anat. u. allg. Path.*, 1928, **81**, 391.

[2] Bürkle-de la Camp, *Deut. Z. f. Chir.*, 1929, **220**, 31.

[3] Hoelzel and Kleitman, *Arch. Int. Med.*, 1927, **39**, 710.

We first starved 35 rats every other day or during alternate 2-day periods and gave diets differing in their protein content to the various rats on feeding days. As a result, some rats seemed to develop ulcers in the prostomach regardless of the type of diet, but practically all rats that were intermittently starved and were fed diets low in protein for more than 2 weeks developed ulcers. No ulcers were found in the prostomach of any of 175 control rats.

The experimentation was therefore extended to determine the effect of more prolonged starvation, of protein restriction without starvation, and of adding salt, spices, alcohol, HCl or ant-acid salts to various diets. /The effect of the major vitamin deficiencies, of high and low carbohydrate, or fat, or protein diets, and of various amounts and kinds of roughage in the diets was also investigated. In nearly all cases, 8 or more rats were used in determining the effect of each specific experimental procedure. Altogether over 1000 rats were used, besides the 175 controls referred to above.

The results primarily confirmed the belief that ulceration could be produced in the prostomach of rats by simple protein restriction. The most direct way of producing ulcers proved to be prolonged starvation and the most striking ulceration occurred when starvation or protein restriction was complicated by pregnancy. Some starvation or undernutrition was practically always involved when ulcers developed. Nevertheless, it could be shown that inanition or undernutrition *per se* was not responsible for the ulcers. For instance, when an exclusive diet of bran was given, no ulcers developed in the prostomach although the rats then practically starved to death in spite of eating enormous amounts. In fact, ulcers produced by starvation or protein restriction would heal on diets of practically nothing but bran. Incidentally, this indicates that mechanical irritation neither gave rise to the ulcers nor seriously interfered with their healing. Presumably, the effect of bran, in this connection, is explained partly by its acid-binding power and partly by the diluting effect of the bulk. The saliva swallowed in ingesting bran may also be a factor.

The ulcers produced in the prostomach of rats by protein restriction were similar to those described by Pappenheimer and Larimore[5] and attributed, by them, to the ingestion of hair. They found ulcers in about 61% of rats kept on diets like the Sherman-Pappenheimer rickets-producing diet, which contains 95% patent flour. Among 27 rats kept on a diet of white bread only, in our study, about two-thirds developed ulcers. The protein inadequacy involved in such diets is evidently responsible for the lesions. Our observations also indicate that the ingestion of hair leads to ulceration only when this interferes with a normal food intake. On the other hand, ulcers do not develop, even when the diet is somewhat poor in its protein content, provided that large amounts are eaten. Also, when from 5% to 10% of an ant-acid salt was added to bread, the food intake decreased and ulceration increased above what occurred on a diet of bread only, but, when from 15% to 30% of calcium carbonate was added to bread, the incidence of ulceration again decreased in spite of a low food intake, apparently because of the great excess of ant-acid salt. Rats on diets high in fat but low in protein developed ulcers, but not as readily as rats kept on high carbohydrate diets with a similar protein content. Rats on diets with as much as 90% protein did not develop ulcers, even when some HCl was added.

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[4] Hoelzel, *Am. J. Physiol.*, 1926, **77**, 166.

[5] Pappenheimer and Larimore, *J. Exp. Med.*, 1924, **40**, 719.

These observations indicate that a protein deficiency or the lack of sufficient acid-binding protein in the diet may be a factor in the etiology or in the chronicity of some cases of gastric or duodenal ulcer in man. The trial of a diet rich in protein in ulcer cases was suggested 5 years ago (Hoelzel[4]) and the recent advocacy of the use of mucin by Fogelson[6] seems to be a step in this direction. However, clinical experience and some experimental observations on man (Hoelzel and Kleitman[3]) and on rats in this study seem to indicate that an excessive protein intake immediately after more or less prolonged restriction may lead to a temporary aggravation of the symptoms or conditions due to protein restriction. Moderation in the protein intake in the early stages of ulcer healing is therefore suggested and this conforms with standard clinical practice. Prolonged protein restriction, however, is definitely contraindicated.

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[6] Fogelson, *J. Am. Med. Assn.*, 1931, **96**, 673.

5895

Profound Morphological Changes Following Ulceration in the Prostomach of Rats.

FREDERICK HOELZEL AND ESTHER DA COSTA.
(Introduced by A. J. Carlson.)

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After finding that ulcers could be produced in the prostomach of rats by protein restriction or starvation (Hoelzel and DaCosta[1]) our attention was directed toward determining whether such ulcers might become malignant. Marked overgrowths were seen early in ulcerated areas, but, as the work of Bullock and Rohdenburg[2] indicated, this is not a malignant change. Simple overgrowths were particularly prominent in the prostomach of rats in which ulceration was produced with diets high in fat and low in protein. This corresponds to the results obtained by Fujimaki and his coworkers,[3] except that these workers believed that they were dealing with cancerous changes due to disturbances in nutrition and independent of ulceration.

The first indication that the changes were like Fibiger's[4] came when a young rat died after 58 days on a diet of white bread. The prostomach showed numerous small nodular prominences on the outer surface. On the inner surface there was some ulceration but with only moderate overgrowth. Experiments were undertaken to produce similar or greater gross changes in other rats. Distinct single or multiple nodules were produced in the prostomach of 31 rats and somewhat vague nodules in 9 others. Considering only those rats with distinct nodules, only 2 occurred among about 200 rats on low protein diets for less than 40 days. Eight appeared among 79 rats on similar diets for 40 to 230 days, with or without starvation of less than 15 days. No distinct nodules were seen in any of 10 rats that died after single periods of starvation of from 15 to 20 consecutive days, but 3 out of 4 rats that died after starving from 26 to 29 days showed nodules. The other 18 cases appeared among 38 rats starved from 2 to 12 times, 5 days or more each time, for periods totaling from 23 to 102 days, and generally with adequate diets in between starvation periods. Seven rats here included had been sacrificed after having been kept long enough on adequate diets, following periods of starvation, to heal all or nearly all ulcers. The object was to determine what effect this would have on any nodules that might be present. In one of the 7 rats, no nodules were evident and the animal may never have had any. In another rat, one nodule was evident on the outer surface of the prostomach and about a dozen were distinct only on the inner surface. In this rat, one large ulcer had not entirely healed. In the other 5 rats, all ulcers had healed, no nodules showed on the outer surface and only from one to 3 nodules were evident on the inner surface of the prostomach in each rat. These results indicate that most, if not all, nodules would eventually disappear with an adequate diet but that they disappear much more slowly than prostomach ulcers.

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[1] Hoelzel and DaCosta, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 382.

[2] Bullock and Rohdenburg, *J. Cancer Res.*, 1918, **3**, 227.

[3] Fujimaki, Okabe, Kimura, Wada, and Hiratsuka, *Trans. Jap. Path. Soc.*, 1928, **18**, 548.

[4] Fibiger, *Z. f. Krebsforsch.*, 1920, **17**, 1.

Histological examination proved that some of the largest nodules were only epithelial cyst-like structures which were generally located at the ridge between the prostomach and main stomach—precisely where Fibiger found cysts. Other nodules were cornified globular masses more or less completely surrounded by epithelium. In most cases a continuity with the normal epithelial layer of the prostomach could be demonstrated. In some rats, small epithelial masses appeared to be separated but this was evidently an artefact due to cutting through atypical downgrowths. In no case was the muscular wall of the prostomach penetrated or invaded, as in Fibiger's rats. On the whole, our results may be explainable on the basis of the views expressed by Bullock and Rhodenburg. They regarded lesions like those produced by Fibiger as probable consequences of simple mechanical and/or chemical irritation. In our work the irritant was the acid gastric juice which primarily produced the ulcers. In the results obtained by Fibiger, Bullock and Rhodenburg, and Fujimaki and his coworkers, the acid gastric juice may also have been the main factor since they all employed diets low in protein or instituted procedures that would interfere with a normal food intake. Whether true malignancy can be produced by a more prolonged application of simple principles such as we employed still remains an open question.

5896

Elimination of Thyroglobulin from the Blood.

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Since thyroglobulin has been detected in thyroid venous blood and lymph[1, 2] but not in the general circulation, it would appear that the compound must be destroyed, or eliminated from the blood stream rather rapidly. At any rate the concentration of the protein does not become large enough to detect by the precipitin method. A study was undertaken to see how rapidly thyroglobulin could be eliminated when injected intravenously, and what the fate of the compound was.

Thyroglobulin was prepared from fresh hog thyroids by precipitating the protein in the isoelectric range. Antibodies were developed in rabbits by injecting this protein, and the antiserum was collected after 10 days. Suitable amounts of the hog thyroglobulin were injected intravenously into dogs. Samples of blood were withdrawn before and at various intervals after injection. Each sample of serum was diluted in a series of tubes and the antiserum added to each. The last tube showing a precipitin test was designated +, and those preceding were roughly gauged ++, +++, and ++++ by inspection.

Table I is a typical experiment.

TABLE I.

Time	Tube 1	2	3	4	5
0	—	—	—	—	—
10 min.	+++	+++	++	+	—
60 "	++	++	+	—	—
90 "	++	+	+	—	—
120 "	+	+	—	—	—

It can be seen that thyroglobulin is steadily removed from the blood stream. Similar results have been obtained with 8 dogs. Smaller dogs remove the protein slower but the rate in terms of body weight has not yet been determined. The rate of elimination is apparently independent of the concentration in the blood. If the time

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[1] Hektoen, Carlson and Schulhof, *J. Am. Med. Assn.*, 1923, **81**, 86.

[2] Hicks, *J. Physiol.*, 1926, **62**, 198.

necessary for 30 mg. of thyroglobulin to disappear is determined, it has been found that injection of 60 or 90 mg. requires about 2 or 3 times longer. One animal has been injected 13 times over a period of 18 days without any perceptible change in the rate of elimination.

It must be remembered that hog thyroglobulin injected into dogs is a foreign protein. This may account in part for the rapid rate of removal. The rate will be determined using dog thyroglobulin. In the above experiment about 60 mg. were removed in 2 hours. This is equivalent to more than 1 gm. of Armour's desiccated thyroid. There are several possibilities to account for the thyroglobulin: 1. The protein as such may be taken up by the tissues. 2. Excretion might occur through the urine, bile or intestine. 3. The protein might be broken up into smaller fragments and these remain circulating in the blood. 4. These fragments might be taken up by the tissues or excreted. The fate of thyroglobulin is being investigated.

5897

Relationship Between Lipase and Neurotoxic Action of Dog's Serum after Experimental Liver Damage

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On the basis of his finding of myelinolytic and lipolytic activity in the sera of patients with multiple sclerosis, Brickner[1] has suggested the therapeutic use of quinine in this condition, since it is known that quinine inhibits the action of some lipolytic enzymes *in vitro*. Weil[2] has confirmed the presence of a neurotoxic agent in the sera of these patients and has also demonstrated that the urine possesses a similar action. Crandall and Cherry[3] have shown that the majority of sera from cases of multiple sclerosis contain an abnormal lipase, similar to that of the pancreas; that diastase is also increased; and that similar findings are present in cases of liver disease and in animals with experimental hepatic damage. It seemed of value to determine whether a neurotoxic agent was present in experimental hepatic damage, and if so whether its appearance and concentration ran parallel to that of lipase.

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[1] Brickner, R. M., *N. Y. State J. Med.*, 1931, 1.

[2] Weil, A., *J. Am. Med. Assn.*, 1931, **97**, 1587.

[3] Crandall, L. A., Jr., and Cherry, I. S., *Arch. Neur. and Psych.*, in press.

Six dogs with complete obstruction of the common bile duct, 5 with ligation of both pancreatic ducts, and 3 with Eck fistulae were studied. Ligation of the pancreatic ducts has been shown by Berg and Zucker[4] to produce fatty degeneration of the liver. For determination of neurotoxic action the sera from these dogs were incubated with rat spinal cord (5 cc. serum with 0.05 gm. cord for 20 hrs. at 37°C.) After 3 days' formalin fixation the spinal cords were imbedded and sections stained for myelin (Weil's method), axis cylinders (Davenport's method) and with cresyl violet. Parallel determinations of lipase were made by the method previously reported.[5]

Normal serum produces only a mild swelling of the nerve fibers in the outer zone of the spinal cord. The serum from Eck fistula dogs was not active on rat cord *in vitro,* but beginning on the fourth or fifth postoperative day sera from animals with ligation of common bile duct or pancreatic ducts produced a severe destruction of both myelin sheaths and axis cylinders. The glia nuclei in the outer zones had lost their staining qualities and the ganglion cells showed marked vacuolation and shadow formation. The abnormal lipase usually appeared in the sera in large amounts within 24 hours after the operation, and had usually reached its peak before neurotoxic activity appeared. The lipase usually disappeared about a month after duct ligation but at this time the neurotoxic activity was still strongly present. Further, it was found that serum heated to 62°C. for 30 minutes lost all lipolytic action but the neurotoxic agent was unaffected.

We conclude that in experimental hepatic damage a neurotoxic agent is present in the blood serum, and that this activity cannot be accounted for by the lipase which is often simultaneously present.

5898

New Methods for Studying Motility During Sleep.

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The methods heretofore employed for investigating motility during sleep involved the continuous recording on a kymograph of the position of the bed-spring or the whole bed, by direct transmission to a writing lever, pneumatic transmission to a recording tambour, or electrical transmission to a signal magnet. The first 2 of these methods furnish a record of the frequency as well as of the magnitude of movement, the last one, only of frequency.

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[4] Berg, B. N., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 68.

[5] Crandall, L. A., Jr., and Cherry, I. S., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 570.

Although it has been shown repeatedly that one stirs and turns over several times per hour during sleep, it has never been determined how much *time* one spends in these movements, or by difference what part of the time given to sleep one really lies still. One of the new methods was developed to answer this question. A large tambour (one foot in diameter) was placed under the bed and connected to the bed-spring by means of a vertical rod. The tambour was supplied with inlet and outlet valves and wired in such a manner that the opening of either valve would break an electric circuit. When the sleeper moved the displacement of the bed-spring downward compressed the air in the tambour causing the outlet valve to open, and the displacement of the bed-spring upward rarified the air causing the inlet valve to open. The valves remained open, and the circuit broken during the entire movement. Through a relay in the circuit a Telechron electric clock was started and kept running during the movement, or else a clock that was running stopped and kept still. With 2 clocks both of these effects could be produced simultaneously. The first clock having been set at zero (12 o'clock) before going to bed, its reading next morning gives the time spent in movement during the night. The same result could be obtained by noting how much time the second clock lost in the same period of time. The inertia of the clock motor did not permit it to respond instantaneously to the make and break of the electric circuit, so that the first clock gave a reading that was too high and the second clock a reading that was too low. It was necessary to calibrate the clocks by recording repeated movements of the bed-spring, of longer and shorter duration, by means of a signal magnet on a very rapidly moving kymograph and comparing the time actually spent in movement with the readings of the clock. One clock is sufficient for the determination of the time spent in movement, but by the use of 2 clocks it is possible to obtain additional information concerning the duration of individual movements.

The sleep of 3 persons has been studied. The time spent in movement was very small, varying somewhat in the different subjects, and in the same subject during different nights. Subject C. spent from 34″ to 87″ and an average of 57″ per hour in movement during sleep (based on 12 nights); subject M. from 13″ to 28″ and an average of 21″ (11 nights); subject K. from 28″ to 79″ and an average of 49″ (16 nights). Concomitant records obtained by the older method showed the motility of all 3 subjects to be within the usual variation as regards frequency of movement. Thus, even though one moves very often, one still lies immovably about 59 minutes out of every hour.

The second method developed is concerned with the sum-total of the displacements of the bed-spring during sleep. A thread attached to the rod connecting the bed-spring with the tambour is fastened at the other end to the lever of a Harvard "work-adder". The wheel of the work-adder can move only in one direction, and the movement of its rim is equal to downward displacement of the bed-spring. The work-adder operates a counter, and by reading the counter it is possible to determine the number of times that the wheel turned around, and thus the sum-total of the displacements of the bed in one direction.

So far 2 subjects were studied by means of the work-adder. The sum-total of the displacements of the bed-spring in the case of subject C. varied from 72.8 to 139.5 cm. (6 nights); for subject M. the figures varied from 43.7 to 97.0 cm. (6 nights). Since the bed-spring returns to the original position, one must double the above figures to obtain the sum-total of all the displacements of the bed-spring.

The methods described are superior to the older ones in that no recording devices and kymograph are needed. The apparatus may be placed under the bed in an ordinary room in a private dwelling that is supplied with a source of alternating current regulated to run electric clocks. The figures for the time spent in movement can be read off directly from the clock and corrected by the factor, and the sum-total of all the movements can be obtained from the readings of the counter attached to the work-adder.

I am greatly indebted to Mr. N. R. Cooperman and Mr. F. J. Mullin for their assistance in the development of these methods, and to Mr. G. Lutz, the departmental mechanic, for his cooperation in the design and the building of the apparatus required.

5899

Effect of Bronchial Stenosis on Pulmonary Tuberculosis in Dogs.

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In a former publication a method for the production of massive atelectasis in dogs was described, consisting of an application of a 35% solution of silver nitrate to the mucous lining of a bronchus. The bronchial lumen subsequently became completely stenosed and was accompanied by massive atelectasis of the obstructed pulmonary lobe. It has long been known that rest of the lung by "collapse therapy" is the method of choice in the treatment of pulmonary tuberculosis. Thus, it was decided to determine the effect of atelectasis on pulmonary tuberculosis in animals. Two groups of dogs were used.

Group I. 18 dogs were subdivided into two parts. In 3 animals a saline suspension (0.1 mg. per kg. of body weight) of a one-month-old culture of human tubercle bacilli was injected into the femoral vein following the production of massive atelectasis, of part of the lung. These were sacrificed at the end of 2, 4, and 8 weeks respectively. In the remaining 15 animals a saline suspension (0.1 mg. per kg. of body weight) of a 4-weeks-old culture of human tubercle bacilli was injected into the femoral vein. Massive atelectasis was produced in 2 of the pulmonary lobes 2 to 4 weeks after injection. Most of this group died or was sacrificed within 2 to 4 months after infection.

Group II. In 8 animals a saline suspension (0.2 mg.) of a 4-weeks-old culture of human tubercle bacilli was injected into the left pulmonary artery through a thoracotomy opening. Massive atelectasis of the entire left lung was produced 2 to 6 weeks after the injection. Two of this group have died; the remaining 6 are living 10 weeks following infection.

Results. Group I (a). No gross tubercles were seen at the 2-week stage. At the end of 4 weeks many tubercles were seen in the inflated lobes. The surface of the atelectatic lobe was finely granular. By 8 weeks many tubercles were found in the inflated lobes and few or none in the atelectatic lobes.

Group I (b). In the early stages, 4 to 6 weeks following infection, the inflated lobes contained many tubercles ranging from 2 to 3 mm. in diameter. The atelectatic lobes at this period contained a few pin-point sized tubercles. In the later stages, 10 to 13 weeks following infection, the inflated lobes were studded with tubercles 3 to 4 mm. in diameter; the atelectatic lobes having an occasional pin-point sized tubercle or none at all.

Group II. One dog died 5 weeks following infection, no lobes having been collapsed by that time. The lobes injected presented many tubercles 1 to 2 mm. in diameter. The opposite lung presented no gross tubercle formation. The second dog died 10 weeks following infection and 5.5 weeks after production of atelectasis. The atelectatic lobes (the side of injection) presented no gross tuberculosis. The inflated opposite lung revealed many large tubercles 2 to 4 mm. in diameter. The microscopic appearance confirmed the gross pathology.

Conclusions. In these experiments little difficulty was encountered in producing pulmonary tuberculosis in dogs by the hematogenous route. Definite marked improvement, to a complete cure of the lesion was obtained by the production of massive atelectasis of the region involved. This improvement or cure in one location was brought about while the animal was dying from infection in another part.

5900

Influence of Vitamin A Deficiency upon Intestinal Permeability for Bacteria.

E. SEIDMON AND LLOYD ARNOLD.

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White rats were fed a diet of casein, cornstarch, salt mixture, dried yeast and butter. In the vitamin A deficiency series the butter was omitted. There were 18 animals in each series and the feeding experiment lasted for 9 and 10 weeks. After 24 hours' fasting 3 billion *B. typhi murii* were administered by stomach tube. Nine animals were killed in an illuminating gas chamber after one half hour and 9 after one hour in both the normal and the avitaminosis series. Specimens of liver and lung, the spleen and one kidney were removed and macerated in broth. Subcultures were made on Endo and plain agar plates after 3, 6, 9, and 24 hours' incubation. One-tenth cubic centimeter of organ-broth suspension was used for

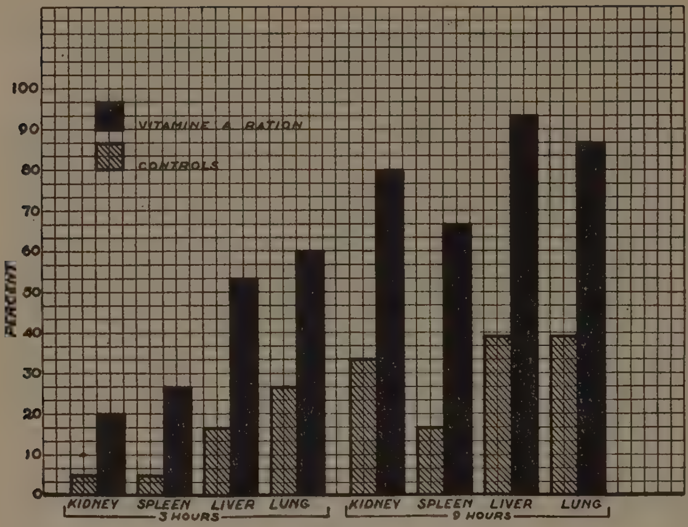

FIG. 1.

Ordinate—% of rats showing presence of *B. murii* in organs.

Abscissa—kidney, spleen, liver and lung macerated in broth and subcultured after 3 and 9 hours' incubation.

Solid column represents vitamin A deficient rats.

Barred column represents normal controls.

subculture. These time intervals were chosen to obtain information upon the relative concentration of the test bacteria in the organs of the animals. The accompanying graph illustrates averages of 18 control and 18 vitamin A deficiency rats at the periods of 3 and 9 hours' incubation of the organ cultures. There are a greater number of animals showing the presence of the test micro-organisms in the organs in the vitamin deficient diet than in the controls at the time intervals of one-half hour and one hour after oral administration. Quantitative studies of the bacteria in the organs will be reported later.

5901

Low Intestinal Obstruction with Biliary, Pancreatic, and Duodenal Secretions Short-Circuited Below Obstructed Point.*

HILGER PERRY JENKINS AND NORMAN W. ROOME.
(Introduced by D. B. Phemister.)

From the Department of Surgery, University of Chicago.

In a previous communication one of us (H. P. J.[1]) found that high intestinal obstruction with the biliary, pancreatic, and duodenal secretions short-circuited below the obstructed point was compatible with life for 12-33 days, with considerable changes in the blood chemistry over this period.

In the present experiments the obstruction was produced in the lower ileum 30-70 cm. from the caecum. The animals were prepared for the obstruction by a preliminary operation in which the pylorus was resected and gastro-jejunostomy performed. At a second operation the jejunum just beyond the ligament of Treitz and proximal to the gastro-jejunostomy was sectioned and the proximal end anastomosed to the lower ileum. The ileum just above this anastomosis was also sectioned. The free sectioned ends of the bowel were invaginated with purse string sutures. In this way the biliary, pancreatic, and duodenal secretions emptied into the lower ileum, and the stomach drained into the long obstructed loop of jejunum and upper ileum.

In 7 dogs the length of life after the second operation varied from 2 to 20 days, averaging 10 days. Vomiting occurred about every 2nd or 3rd day. The animals took fluids by mouth fairly well. The urine output averaged 150-350 cc. per day. Studies of the blood chemistry showed only a moderate fall in the chlorides in a few instances, whereas in several it was practically the same as before operation. Carbon-dioxide combining power did not show any constant marked change. The non-protein nitrogen was inclined to rise in several of the animals. At autopsy the stomach showed slight distension, the characteristic finding being a marked dilatation of the terminal portion of the obstructed bowel, which in some animals was 5 cm. in diameter. The proximal portion of the obstructed bowel showed relatively slight distension. The cause of death was definitely due to a complication in 4 ani-

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research.

[1] Jenkins, H. P., *Arch. Surg.*, 1929, **19**, 1072.

mals; however, in 3 cases no cause other than the presence of the obstruction could be demonstrated at autopsy.

In comparing this experiment with the high obstruction previously described,[1] the length of life is considerably shorter in this series, the blood chemistry shows relatively slight changes, and the obstructed bowel is more markedly distended in the terminal portion. The length of life is approximately the same as observed in animals with ordinary obstruction at this level without short-circuiting the 3 secretions.[2] The relatively slight changes in the blood chemistry agree with other observations on animals with ordinary low obstruction.[2]

From these observations we feel that death in low obstruction cannot be explained primarily on the basis of loss of digestive secretion, and if death in this type of obstruction is due to the elaboration and absorption of a toxic substance in the obstructed bowel, the presence of the biliary, pancreatic, and duodenal secretions is not necessary for this process to occur and to cause its usual fatal outcome.

5902

The Absence of Gonad-Stimulating Hormone in the Urine and Blood of Patients with Pituitary Tumors.

JAMES W. WATTS. (Introduced by H. B. van Dyke.)

From the Division of Neurology and Neurosurgery of the University Clinics, University of Chicago.

The gonad-stimulating effects of anterior pituitary implants were demonstrated independently by Smith[1] and by Zondek and Aschheim.[2] This was soon followed by the discovery of Zondek and Aschheim[3] of a gonad-stimulating hormone in the urine of pregnancy. It was thought that a similar substance might also be present in the urine or blood of acromegalics. Probstner[4] includes 2 acromegalics in a series of obstetrical and gynecological patients on whom he made the Aschheim-Zondek test, and simply remarks that both gave a negative reaction.

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[2] Elman, R., and Hartmann, A. F., *Surg., Gynecol., and Obstetrics*, 1931, **53**, 307.

[1] Smith, P. C., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 131.

[2] Zondek, B., and Aschheim, S., *Arch. f. Gynäk.*, 1927, **130**, 1.

[3] Zondek, B., and Aschheim, S., *Klin. Woch.*, 1928, **7**, 831.

[4] Probstner, A. v., *Endokrinologie*, 1930, **7**, 161.

Experiments were made in the attempt to demonstrate the gonad-stimulating hormone in the urine and blood of patients with pituitary tumors. The 14 patients, of whom 7 were men and 7 women, may be divided into 4 groups. The first 2 (Cases I-VI) are characterized by typical acromegalic features, hands and feet, enlargement of the sella turcica, and tufting of the distal phalanges. The first group (Cases I-III) has signs of activity such as profuse perspiration or marked weakness, and normal or elevated basal metabolic rates. The second (Cases IV-VI) has no such evidence of activity and definitely lowered basal metabolism. The third group (Cases VII-XI) is characterized by the absence of acromegaly, and the presence of amenorrhea or impotence, visual disturbances, enlargement of the sella turcica and low basal metabolism. The last group (Cases XII-XIV) consists of "suspects" who have headaches and changes in the sella turcica.

The urine was preserved with 0.2% metacresol and kept at icebox temperature and the blood was kept at the same temperature but no preservative was used. Rats were given 4 daily injections of ¼ the amount of each total dose beginning on the twenty-first day and sacrificed on the twenty-sixth day, 120 hours after the first injection, following the method of assay of Wallen-Lawrence and van Dyke.[5] Quantitative evaluation of the potency of the material tested was based on the weight of both ovaries free from bursae and fallopian tubes. These workers found that the mean weight of the ovaries in a series of controls was 16.14 mg. and calculated the upper limit of normal to be 26.3 mg. When male rats were used the seminal vesicles were weighed; in their controls the mean weight was 9.24 mg. and the upper limit of normal calculated to be 14.9 mg. In my series of controls the mean weight of the ovaries was 13.7 mg. and of the seminal vesicles 7.5 mg. Therefore, ovarian weights equal to or exceeding 26.3 mg. and seminal-vesicle weights equal to or exceeding 14.9 mg. were considered significantly enlarged. The results of the assays of 100 rats are given in the accompanying tables. As can be seen none of the mean weights of the ovaries or seminal vesicles exceeds the upper limits of normal. Although not shown in the tables, the weight of no single pair of ovaries or seminal vesicles was as great as 26.3 mg. or 14.9 mg., respectively. In no case were ruptured Graffian follicles, corpora lutea, oestrous smears or vaginal canalization present. Uterine hypertrophy was present in one rat killed on the thirty-third day (Table I, Case VI). The urine of cases III and V killed the rats in from 12 to 48 hours. The urine of Case III contained many colon bacilli; it was extracted with ether; the alcoholic precipitate (75%) from the ether-extracted urine was then injected in an amount equivalent to 24 cc. In

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[5] Wallen-Lawrence, Z., and van Dyke, H. B., *J. Pharm. Exp. Ther.*, 1931, **43**, 93.

TABLE I.
Cases of Acromegaly.

Case	Material Injected	Total Dose	No. of Females	Mean Wgt. of Ovaries	No. of Males	Mean Wgt. of S. V.	Remarks
		cc.		mg.		mg.	
I (F)	Urine	1.0	1	10.4	—	—	Subcut.
,,	,,	4.0	1	10.4	4	6.4	,,
,,	Serum	3.0	1	13.7	—	—	Intraperit.
,,	,,	6.0	2	17.0	—	—	,,
II (M)	Urine	4.0	2	12.1	2	9.7	Subcut.
,,	,,	14.0	1	11.6*	3	9.8*	Intraperit.
,,	Defibrinated Blood	4.0	1	15.7	2	11.9	,,
III (M)	Urine	24.0	2	14.4	4	10.2*	Intraperit.
,,	Defibrinated Blood	4.0	2	14.3	1	12.2	,,
IV (F)	Urine	4.0	2	11.7	2	6.6	Subcut.
,, (F)	,,	12.0	2	10.1*	2	6.1*	Intraperit.
,,	Defibrinated Blood	3.0	2	18.4	—	—	,,
VI (M)	Urine	1.0	1	13.2	—	—	Subcut.
,,	,,	4.0	2	14.9	2	6.6	,,
,,	,,	14.0	1	20.8*	2	7.6*	Intraperit.
,,	Defibrinated Blood	3.0	2	16.1	1	8.0	,,

* These rats were given 1 cc. of urine subcutaneously from the twenty-first to the twenty-third day and the remainder intraperitoneally from the twenty-sixth to the thirty-first day and killed on the thirty-third day.

TABLE II.
Cases of Chromophobe Adenoma.

Case	Material Injected	Total Dose	No. of Females	Mean Wgt. of Ovaries	No. of Males	Mean Wgt. of S. V.	Remarks
		cc.		mg.		mg.	
VII (F)	Urine	4.0	3	16.3	1	8.6	Subcut.
VIII (F)	Urine	1.0	5	15.1	1	8.4	Subcut.
,,	,,	4.0	2	16.1	4	7.7	,,
,,	Serum	5.0	2	18.5	—	—	Intraperit.
,,	,,	7.5	2	15.4	—	—	,,
IX (F)	Defibrinated Blood	4.5	2	17.8	2	10.7	Intraperit.
,,	Serum	4.0	2	16.0	—	—	,,
X (M)	Urine	8.0	1	11.6	1	8.3	Intraperit.
XI (F)	Urine	4.0	3	16.1	1	8.7	Subcut.
	,,	10.0	1	16.0	3	8.1	Intraperit.

TABLE III.
Cases of Possible Pituitary Adenoma.

Case	Material Injected	Total Dose	No. of Females	Mean Wgt. of Ovaries	No. of Males	Mean Wgt. of S. V.	Remarks
		cc.		mg.		mg.	
XII (M)	Urine	4.0	2	9.4	2	6.6	Subcut.
"	"	12.0	—	—	4	9.0*	Intraperit.
XIII (M)	Urine	4.0	2	16.8	2	8.2	Subcut.
	"	12.0	1	15.2*	1	13.6*	Intraperit.

* These rats were given 1 cc. of urine subcutaneously from the twenty-first to the twenty-third day and the remainder intraperitoneally from the twenty-sixth to the thirty-first day and killed on the thirty-third day.

all other cases undiluted urine was used. The urine of Case XIV was injected subcutaneously into 7 rats; each of 4 injected with 1 cc. and one injected with ¼ cc. died in 12 hours. Two others given ¼ cc. appeared sick, failed to gain weight, and the site of the infection became indurated and adherent to the underlying muscles. The experiments on urine from Cases V and XIV are, therefore, not included in these tables.

These experiments show that no gonad-stimulating hormone was demonstrated in the urine or blood of patients with pituitary tumors by the injection of as much as 24 cc. of urine or 7.5 cc. of blood serum.

5903

A Bacteriophage Feces Media.

A. BART. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

It became desirable in the course of some investigations to develop a media to determine the relative biological stability of fecal *B. coli* strains to bacteriophage. The relative sensitiveness of the *B. coli* flora can be altered by diet. The isolation of colonies and testing their phage-sensitiveness is too involved for practical purposes. We wished to develop a media on which we could plate our fecal specimens and read directly the percent of *B. coli* flora resistant and susceptible to bacteriophage.

Endo, Eosin Methylene Blue, Conradi-Drigalski and Teagues media were used. Fifty cc. of these various agar media were put in flasks and sterilized. After cooling to 50°C. Coli-phage was added in amounts varying from 0.1 cc., 0.3 cc., 0.5 cc., 0.7 cc., 1 cc. and 2 cc. and poured into petri dishes. The surface was seeded and smeared with spreader, then incubated for 21 hours. The best results were obtained when 0.3 cc. and 0.5 cc. was added to the 50 cc. agar media. Increase in concentration of phage causes inhibition of *B. coli* growth. The eosin methylene blue media has worked best in our hands. These plates must be protected from light. The phage is destroyed or inhibited when exposed to light. Eosin Methylene Blue phage media can be stored for days in the dark in the icebox and retain its original phage content. The *B. coli* susceptible colonies lose their shining luster, are smaller and irregular. A relatively clear, transparent zone develops around the *B. coli* phage-sensitive colonies. When viewed against a light background the ratio of phage-resistant to phage-sensitive *B. coli* colonies can be readily determined.

5904

Influence of Vascular Impairment on Absorption of Bacteria into Blood from Upper Intestinal Tract.

F. A. CIRRINCIONE AND A. FRANCONA. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

Vercellano found that there was absorption of bacteria during 24 hours, following ligation of the superior mesenteric vessels to the duodenum. His technic consisted of ligating both the superior mesenteric artery and vein, and withdrawing specimens of blood directly from the rabbit's heart at time of ligation and after 1, 4, 12, 18, and 24 hour intervals culturing this blood in broth for 24 hours. His conclusions were that the bacteremia present was due to the lack of blood supply. However, he failed to test absorption of bacteria within intervals of less than one hour.

Twenty-five dogs were used for this experiment under nembutal anesthesia (3% solution, using 1 cc. per kilo). In the control series (8 dogs) a 3-inch midline incision was made in the abdomen. Fifty cubic cm. of a suspension of *B. prodigiosus* (24 hour agar culture using 50 cc. of saline) was injected into the duodenum with a fine needle syringe. At 5 minute intervals, 2 drops of blood were taken from femoral vein and plated on agar.

In the second series (8 dogs) used the above technic and ligated 3 large rami of the superior mesenteric artery to the duodenum before injecting the *B. prodigiosus* suspension. Blood was withdrawn every 5 minutes for one hour and plated on agar.

In the third series (9 dogs) used the above technic with the exception that the blood was withdrawn at intervals for a 2-hour period. (See chart.)

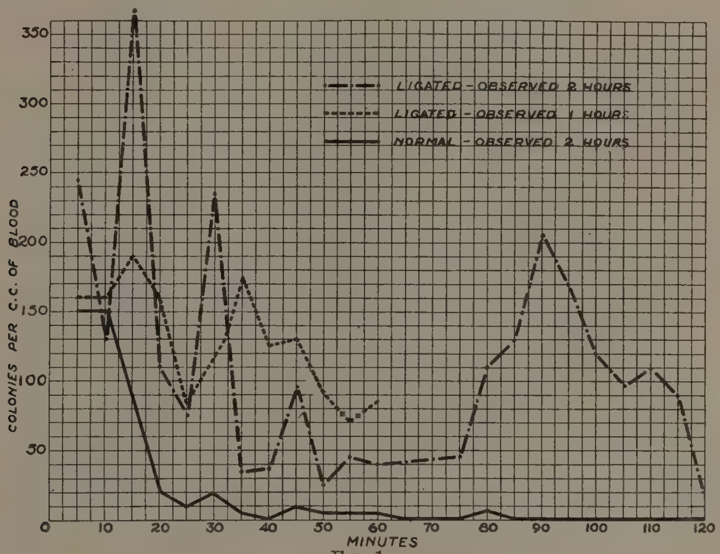

FIG. 1.

Ordinate represents the number of bacteria per cc. of blood from femoral vein. Abscissa represents time in 10 minute intervals.

B. prodigiosus injected into the duodenum and cultures taken from femoral vein; results are represented by continuous line. Same technic, except superior mesenteric artery, ligated, represented by the dot-dash line in one series for 2 hours, the dotted line prolonged for only one hour after injection.

The abscissa represents the time in minutes. The ordinate represents the number of colonies per cc. of blood.

Our results show that in the control series we have some slight absorption from the intestine immediately, while in the other 2 series, there is a marked increase in the rate of absorption.

Therefore, any disturbance of the arterial blood supply of the small intestine (especially the upper portion) causes increase in rate of absorption of bacteria into the blood from the small intestine.

5905

Leucocytic Concentration in Skin and Trachea Following External Application of Heat and Cold.

A. J. NEDZEL. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

Several observers have used alterations in the number of leucocytes in the peripheral circulation as index of the autonomic functional status of the area under examination. This report represents a preliminary study of the influence of the application of heat and cold to the skin of dogs upon the relative concentration of leucocytes in the trachea as compared to the skin.

The trachea of a dog was exposed by median line incision; nembutal anaesthesia (1 cc. of a 3% solution per kilo intraperitoneally) was used. The incision was extended through the wall of the trachea and about 2-inch length of the mucosa was exposed. Blood specimens were taken simultaneously from the mucosa of the trachea and from the ear of the animal for leucocyte counts. These counts were made through a certain extent of time during which ice and hot water packs were applied to the body surface.

The accompanying chart (I and II) shows the result of these experiments. The abscissa represents the time in minutes and the ordinate the leucocyte count. "N" is the average normal leucocyte count, plus and minus representing an increase or decrease of the leucocytes expressed in percent. The continuous line shows the leucocyte count of peripheral blood and the dotted line the leucocyte count of blood from the mucosa of the trachea. Ice packs were applied to the chest and abdomen in 2 animals and the results shown in Chart I. Chart II (8 animals used) represents the same procedure, except that heat (hot moist towels) was applied at the time indicated.

To these 2 series we added (Chart III) (2 dogs) where instead of chilling the animal with ice we injected intravenously 10 cc. of milk. This produced a much stronger reaction.

The alteration in the concentration of leucocytes per unit volume of blood in the capillaries of the ear and of the trachea of dogs indicates that both follow the same general autonomic pattern. The initial response to heat applied to remote skin areas indicates a dilation of the blood vessels in the mucosa of the trachea at the same time as

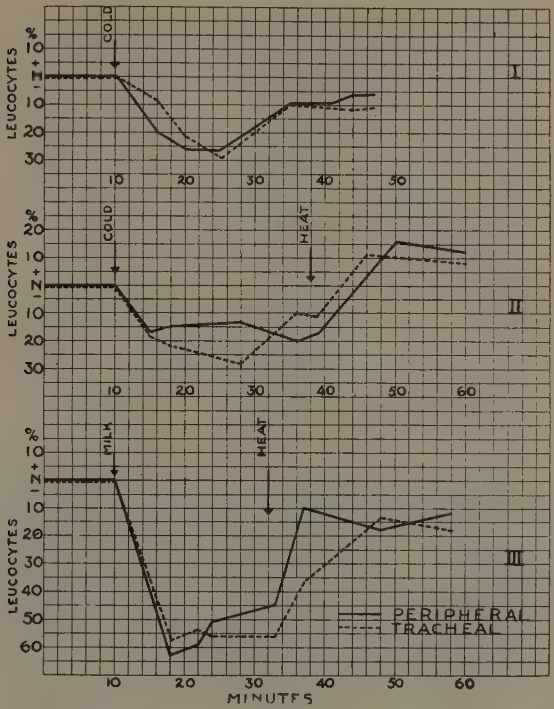

Fig. 1.

Ordinate: Leucocytes per cc. of blood.

"N" represents the normal or beginning count. The % increase or decrease are indicated above or below this initial count.

Abscissa: Time in minutes.

The continuous line represents the peripheral (ear) leucocyte curve, the broken line, the same in blood from mucosa of trachea.

the same reaction takes place in the skin. When cold is applied to remote skin areas, the tracheal capillaries contract with those of the skin.

5906

Motility in the Examination for B. Typhosus and Related Pathogens.

H. E. MCDANIELS, V. M. RYAN AND M. BOYER.
(Introduced by Lloyd Arnold.)

From the Research Laboratories, Illinois State Department of Public Health, and Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine.

Bacteriologic diagnosis of typhoid fever and related infections has centered about the utilization of biochemical differences between the pathogenic organisms concerned and the non-pathogenic forms associated with them. The usual initial procedure in the examination of a suspected typhoid stool is seeding upon some lactose plating medium to eliminate all but the non-fermenting organisms present. The principal reasons to explain the failure to isolate the typhoid bacillus are: scarcity of *B. typhosus* in the stool, or the overwhelming antagonistic action of the associated bacteria. Glycerin, bile, and selectively bacteriostatic substances such as brilliant-green have been incorporated into the stool fluid or into the media. The object is either to forestall the destruction of whatever typhoid bacilli may be present or to hinder the growth of the non-pathogenic organisms of the mixture without inhibiting *B. typhosus.*

Given a mixture in which the desired bacteria were present in very small numbers, it would seem, *a priori,* that instead of inhibitive methods, enrichment would be the procedure of choice. Based upon the conception that, under similar conditions, *B. typhosus* and the Salmonella organisms are more actively motile than *B. coli,* the principal interfering organism, we have experimented with methods of selective enrichment.[1]

Our first attempts with liquid media were only partially successful. Difficulties with diffusion and convection currents during incubation were such as to make the results very irregular. The next trials were with semi-fluid agar in test-tubes; the inoculum was introduced into the bottom of the column of soft agar by a capillary pipette. Disruption of the agar by the pipette allowed rapid ascent of all the organisms in the test mixtures or in feces. Our best results have been with small U-tubes containing ordinary beef infusion to which 0.2% agar has been added. These U-tubes are made of soft glass tubing of an internal diameter of 8 mm. and a thickness of 1 mm. The tubing is cut into 15 cm. lengths and bent into U-tubes after heating over a fish-tail burner. Melted agar is poured into the U-tubes until the column of medium is 4 cm. long. Cotton plugs are put into both ends of the tube, and the outfit is autoclaved. About 0.1 cc. of fecal suspension is placed upon the surface of one end of the agar column, the tube is incubated for 18 hours at 37°C. and then Endo or eosin-methylene blue plates are inoculated with agar removed from the opposite side of the U-tube by means of a sterile wire loop. The more actively motile typhoid and paratyphoid organisms are usually obtainable in predominating numbers and frequently in pure culture. It has been found that the optimum amount of medium for an 18-hour incubation period is a column 4 cm. in length. Evaporation is rapid in these tubes, so that outfits prepared in advance and stored in the ice-box should be examined carefully to insure the presence of an adequate amount of

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[1] See also: Wassen, Anders, *C. R. de la Soc. de biol.*, 1930, **104**, 523.

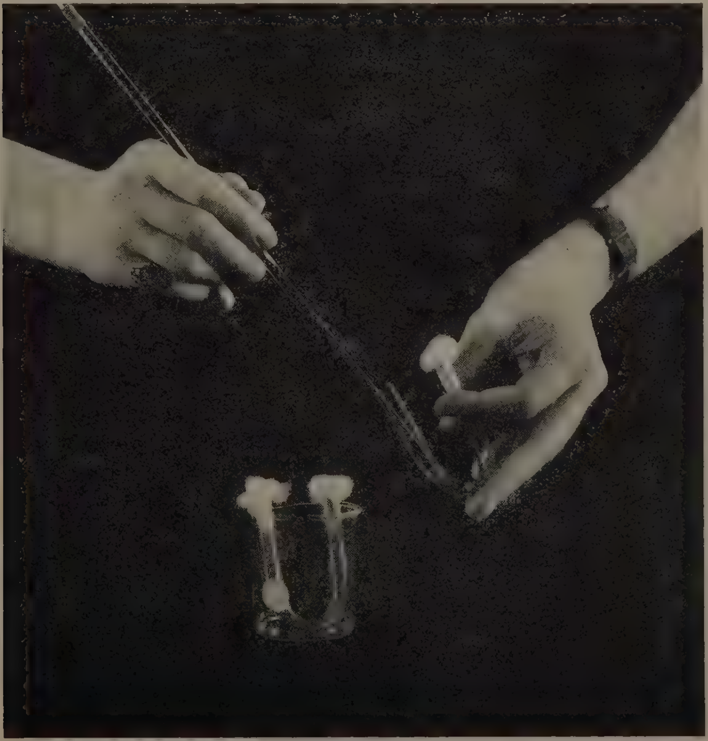

FIG. 1.

medium. Limited application of this procedure to routine fecal specimens has given excellent results and has led in one case to the isolation of *B. paratyphosus B.*, which did not appear on the plates made directly from the specimen.

The table illustrates typical results obtained with dilutions of *B. typhosus* made in a 24-hour broth culture of *B. coli* and plated on Endo by means of glass spreaders.

TABLE I.

Tube	Dilution of *B. typhosus*	Detectable Colonies of *B. typhosus.*	
		Direct plating	From U-tube
1	10^{-1}	29	85
2	10^{-2}	3	all
3	10^{-3}	1	95
4	10^{-4}	2	100
5	10^{-5}	1	90
6	10^{-6}	0	80
7	10^{-7}	0	90
8	10^{-8}	0	0

Tubes 6 and 7 gave no colonies on direct plating, but after growing through the U-tube presented the same result as the lower dilutions. The 1:100,000,000 dilution apparently contained no typhoid bacilli. This method is not offered to take the place of the usual plating of fecal specimens, but to supplement it, and it is, of course, not applicable to the dysentery group of bacilli.

Western New York Section.

University of Buffalo Medical School, December 12, 1931.

5907

Respiratory Metabolism of the Germinating Castor Bean.

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(With the assistance of Jean D. Watkeys and W. R. Murlin.)
From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Many studies of the respiratory metabolism and energy changes in fatty seeds have been made, but none of them completely accounts for the relationships between oxygen absorption and carbon dioxide production observed at different stages of the germination. The present is the first of 3 studies now in progress in this laboratory to secure additional light on the significance of the low respiratory quotients reported by Godlewski[1], Gerber[2], and Ermakov and Ivanov[3].

The castor bean has been chosen for special study because of the nearly total absence of carbohydrate at the beginning of germination. Measurements of the respiratory exchange have been made in many stages from the first appearance of the radicle to a total length of the hypocotyl of approximately 10 cm. Stages up to 30-40 mm. length can be measured conveniently in the ordinary Brodie-Warburg respirometer. Stages beyond this have been studied over periods of 2 to 8 days on moist paper in tightly stoppered bottles covered with opaque paper and kept in the dark. Low quotients are obtained as soon as the growth of the radicle gets well started (length of 1 to 4 mm.).

Attention is directed to only 2 significant results. 1. The wide difference between the R. Q. of the endosperm and that of the young plant inclusive of the cotyledons, which in the castor bean are easily stripped out of the endosperm, and 2. that this difference seems to be due more to the lower CO_2 output of the endosperm than to the larger O_2 absorption. These contrasts are illustrated by the accompanying table, where the O_2 and CO_2 are expressed in cu. mm. per hour per 100 mg. of the whole germinating seed, endosperm alone, and young plant alone, respectively.

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[1] Godlewski, E., *Pringsheim's Jahrb. f. Wis. Botanik*, 1882, **13**, 491.

[2] Gerber, C., *Actes 1st Cong. Internatl. Bot.*, Paris, 1900, 59-101.

[3] Ermakov, I. A., and Ivanov, N., *Biochem. Z.*, 1931, **231**, 79.

TABLE I.
Respiratory Metabolism of Germinating Seed, Endosperm Alone, and New Plant Alone, Calculated to Unit Time and Weight.

No.	Part	Moist wt. mg.	Cu. mm. CO_2/ hr./100 mg.	Cu. mm. O_2/ hr./100 mg.	R. Q.
36	Germinating Seed	911	52.6	133.4	0.394
36	Endosperm	575	53.0	140.9	0.378
36	Young Plant	144	139.0	180.0	0.776
37	Germinating Seed	1047	47.6	104.6	0.454
37	Endosperm	601	53.2	111.1	0.468
37	Young Plant	194	141.5	170.5	0.829

The R. Q. of the young plant when first separated from the endosperm is between 0.9 and 1.0, but it rapidly declines and in the second or third hour reaches the levels shown in the table, probably because of the rapid consumption of sugar. The R. Q.'s obtained by confining from 6 to 24 germinating seeds continuously in darkened, closed bottles for 2 to 8 days from beginning of germination, range from 0.47 to 0.57. These quotients are quite too low to be accounted for by the conversion of glycerol alone to carbohydrate. A possible interpretation is that sugar is formed in the endosperm under the influence of the cotyledons, not only from glycerol but also from the ricinoleic acid, and is transported to the young plant where a part of it is oxidized and a part transformed into cellulose. However, this conclusion cannot be drawn securely until it is known what part in the total process is played by the organic acid discovered by Green.[4]

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[4] Green, J. R., *Proc. Roy. Soc.*, 1890, **48**, 370.

5908

Lowered Resistance to Heat in Adrenal Insufficiency.*

FRANK A. HARTMAN, J. E. LOCKWOOD AND L. M. LOCKIE.

From the Department of Physiology, University of Buffalo.

Gautrelet and Thomas[1] observed that the rapid breathing usually produced by exposure to high temperatures was absent or very much reduced in the late stages of adrenal insufficiency. In our care of adrenalectomized animals in the laboratory, we have found that high temperatures affect these animals easily. When accidentally overheated, they die before the normal animals.

A study has been made of the effect of heat on adrenalectomized rats. When placed in a temperature of 37-40°C. at first both normal and adrenalectomized rats move about the cage. A little later, however, the adrenalectomized animals become quiet and try to sleep, only occasionally moving about. As time goes on the adrenalectomized animals become prostrate while the normal animals show discomfort only by continually moving about the cage and washing themselves with saliva. Frequently the adrenalectomized animals die in a temperature which is not serious for the normals exposed the same length of time. The adrenalectomized animals easily pass into convulsions. At this time convulsions can be brought on by a slight stimulus.

Cortical extract can protect adrenalectomized animals to a considerable degree. If cortin injected and sodium chloride injected (0.5 cc. twice daily of extract and isotonic NaCl solutions respectively) rats which have been adrenalectomized are placed in the same high temperature, the difference between the 2 is much like that between normal animals and untreated adrenalectomized animals except that it is not quite so striking. We are unable at present to explain this increased resistance brought about by cortin.

We have studied the water content of various tissues in the body after exposure to heat.

It will be noted that the water content of the blood and muscle shows the least difference in the various groups. The liver comes next while the skin shows the widest differences. In all instances the cortin treated adrenalectomized rats show a water content between that of normal values and the values of the sodium chloride

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] *Compt. rend. Soc. de biol.*, 1909, **67**, 386.

TABLE I.
% Water in Tissue after Exposure to 38°C.

Group	Blood	Skin	Muscle	Liver	All Tissues	Colonic Temp.	Duration (min.)	Loss wt. (gm.)
Normal (3)	77.9	65.8	76.5	70.7	72.7	40.0°C.	98	5.0
Cortin (7)	79.4	69.8	76.2	71.1	74.1	39.8°C.	110	6.0
NaCl (6)	79.6	72.3	77.6	74.9	76.1	40.6°C.	78	3.6

treated rats. The sodium chloride treated rats were exposed to heat on the average of 78 minutes as compared to 98 minutes for the normals and 110 for the cortin treated. The much shorter period for the sodium chloride rats was due to their inability to resist the heat longer. The higher colonic temperature (40.6°C.) of these rats compared with that of the cortin treated and normals may indicate that they were unable to lose heat as effectively. This may be due in part to a failure of the shift of water through the membranes, because the skin, one of the reservoirs, still retains more water than is found in the normals. Liver likewise contains more. It would seem from these limited observations that less effective mobilization of water is in part responsible for the lowered resistance to heat in adrenal insufficiency.

Minnesota Section.

University of Minnesota, December 16, 1931.

5909

Unilateral Renal Agenesis in a 10 mm. Human Embryo.

EDWARD A. BOYDEN.

From the Institute of Anatomy, University of Minnesota, Minneapolis.

On *a priori* grounds, failure of the kidney to develop has long been attributed to absence of one of its 2 primordia—either the ureteric bud of the Wolffian duct or the metanephric blastema. Recently, in an instructive analysis of defective strains of mouse embryos descended from a pair of x-rayed ancestors, Brown has shown that mechanical conditions which delay or prevent the union of primordia, result in abortive kidneys or total absence of the organ.[1] It appears, however, from her published data, that in a number of cases absence of the organ might be attributed to total absence of a ureter, but there were no cases in which the blastema failed to appear.

This is in accord with the writer's earlier observations,[2] that when downgrowth of the Wolffian duct was interrupted, experimentally, in chick embryos, so that no ureteric bud was permitted to form, the blastema always appeared as a discrete mass of cells; which, however, failed to differentiate into tubules, in the absence of the ureter. Furthermore, from a re-study of these, and hitherto unpublished experiments with chick embryos, it seems that lesions sufficient to interrupt the downward growth of the Wolffian duct, to inhibit the formation of tubules in the lower part of the mesonephros and to injure the spinal cord, are not sufficient to prevent the appearance of the metanephric blastema.

In view of this difference in power of resistance exhibited by duct and blastema, the writer was not surprised when he found a

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[1] Brown, Alice L., *Am. J. Anat.*, 1931, **47**, 117.

[2] Boyden, E. A., *J. Exp. Zool.*, 1924, **40**, 437; PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 572.

10 mm. human embryo in the Minnesota collection in which prospective absence of the left kidney could be explained on the basis of absence of the left ureter. In this specimen, which is being reconstructed for publication, the somewhat dilated left Wolffian duct terminated at the level of the pylorus. On the opposite side, the Wolffian duct had reached the cloaca and had given rise to a ureter which, in turn, had united with the blastema of that side to form the right kidney. Attached to the latter, on its left side, was the rudimentary blastema of what would normally have been the left kidney. In spite of its contact with the good kidney, the left blastema was unable to differentiate into tubules in the absence of the left ureter.

Associated with this anomaly and probably underlying it, was an arrested development of both mesonephroi. Instead of the normal 35 to 38 tubules on a side, the left Wolffian body contained 12 glomeruli, the right one 17. Below the last of the tubules, the Wolffian bodies contained progressively larger posterior cardinal veins—especially on the left side—which, in the region of the umbilical arteries were in contact with the metanephric blastemas and undoubtedly aided in bringing these anlages together in the form of a potential horseshoe kidney.

So far as the writer is aware, this is the first description of the critical stage in the development of unilateral renal agenesis in man. This case, together with the experimental evidence cited, would seem to substantiate the theory that at least one cause of congenital absence of the kidney is lack of formation of the ureter following arrested development of the Wolffian duct and body.

5910

Simultaneous Oscillographic Records of Sound Waves and Electric Variations in the Brain During Avertin Anesthesia.

STARKE HATHAWAY AND GRANT L. RASMUSSEN.
(Introduced by A. T. Rasmussen.)

From the Department of Psychology and Department of Anatomy, University of Minnesota.

In a preliminary experiment under Avertin anesthetic the Wever-Bray experiment was repeated and their findings verified. The apparatus was an ordinary audio-frequency amplifier with head phones. The need for a more accurate record of the impulses from the nervous system was apparent. We first turned our attention to improvements in the amplifier. It was necessary to obtain a maximum of amplification with a minimum number of tubes. To meet this requirement we selected a resistance coupled circuit employing 2 four element type '24 tubes, in the first 2 stages and one type '47 pentode tube as an output. The screen potential of the second tube was varied by a wire potentiometer as a control of amplification. To prevent power loss on the output an output transformer suitable for the pentode was used.

For making permanent records a Westinghouse multi-element moving coil type oscillograph offered most promise of flexibility. The secondary of the output transformer was selected to match the "super-sensitive" strings of the oscillograph. These strings have a sensitivity of approximately .002 ampere per inch deflection with a resistance of 8 ohms. The oscillograph is supplied with 2 of these elements and 2 "standard" elements which may be used for timing or other purposes. Two of the above amplifiers were constructed and checked to make certain that they gave very nearly identical curves on the 2 super-sensitive oscillograph strings. The completed set up affords an undistorted output from below 80 to more than 10,000 cycles, and in the range from 80 to 1000 cycles an input potential of 50 micro-volts will give an output deflection of one centimeter with a quite steady base line and a sufficiently flat response. One amplifier and string were used for the electrical changes from the ear and the other was attached to a microphone pickup so that sounds could be photographed simultaneously from microphone and preparation.

Using the above apparatus we first operated from below, drilling through the base of the skull directly over the trapezoid body, and obtained responses with the active electrode on the surface of the medulla in this region without any injury to the brain tissue. The ear was now stimulated at given frequencies from a loud speaker unit driven by a General Radio oscillator (Type 377B). The responses recorded were strongest in the region of the auditory fibers and corresponded in frequency and in relative amplitude with the input.

We next operated from the dorsal aspect, immediately caudal to the tentorium, damaging as little as possible the cerebellum, placing the uninsulated tip of the active electrode in the region of the caudal portion of the inferior colliculus. In this case there was a definite localization of the point of maximum intensity and that point was on the contralateral side from the stimulated ear. Having set the electrode in this region and obtained records showing the usual frequency correlations, we hardened the brain with the electrode *in situ* and sectioned in order to locate more exactly the position of the exposed electrode tip. This was found to be in the region corresponding to the position of the lateral lemniscus.[1]

From the above animal, using head phones, we were able to hear tones up to 3000 d. v. per second although at too low an intensity for photographing on the oscillograph.

The authors present these data without intending to commit themselves as to their meaning. They offer a more accurate and reliable check on some of the previously published experiments.

5911

A New Colorimetric Method for the Determination of Soluble Fluorides.

WALLACE D. ARMSTRONG. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota.

Soluble fluorides in neutral solution react with Fe^{+++} to give a complex which does not develop a color with the various reagents for iron. Guyot,[1] Greef[2] and Treadwell and Köhl[3] have developed a colorimetric titration method for fluoride based on this fact. Our efforts have been directed towards the application of this principle as a method for the determination of fluorides colorimetrically. We have used thiocyanate, salicylic acid, 8-hydroxy quinoline, and acetylacetone as reagents for the development of color with the excess iron added to a fluoride solution. We have found acetylacetone to be the superior reagent because the others either give colors which are not stable to light, or the amount of fading induced by varying concentrations of fluoride is erratic. Our present method of procedure is as follows: The solution containing the fluoride is rendered just acid to phenolphthalin and diluted to 100 cc. To each of two 25 cc. volumetric flasks is added 1 cc. of a freshly prepared solution of $FeCl_3$ containing 0.3 mg. Fe/cc. and 1 cc. of a

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[1] Davis, H., and Saul, L. J., *Science*, 1931, **74**, 205.

[1] Guyot, *Comptes Rendus*, 1870, **71**, 274; 1871, **73**, 273.

[2] Greef, *Ber.*, 1913, **46**, 2511.

[3] Treadwell and Köhl, *Helv. Chem. Acta.*, 1925, **8**, 500.

0.05% solution of acetylacetone in water. The contents of the flasks are mixed and the red color of ferric acetylacetone develops. To one of the flasks is added 10 cc. of the fluoride solution. (If this aliquot assays over 0.3 mg. fluoride the assay is incorrect and must be repeated with a smaller aliquot; if less than 0.01 mg. a larger aliquot is taken.) The flasks are diluted to volume and the contents mixed. The color of the contents of the flask to which the fluoride was added fades as a consequence of the action of the F^-. The solution containing the fluoride is placed in the right cup of the colorimeter, while the solution containing no fluoride is placed in the left cup and set at 20 mm. A Bausch and Lomb No. 3610 blue color filter is placed in the eyepiece of the colorimeter and a ground glass and 500 watt mazda lamp is placed in front of the mirror. Twenty readings are averaged. This procedure is repeated on the same size aliquot plus a known quantity of fluoride (0.1 mg. F). By plotting colorimeter readings against concentration of fluoride, a straight line was obtained with amounts of fluoride up to 0.40 mg. and agreed with the following empirical formula:

$$F = \frac{0.1\,D\,(X-20)}{Y-X}$$

Where F represents mg. fluoride in the total solution, X is the reading of unknown, Y is the reading of unknown plus 0.1 mg. F and D represents the ratio of the total volume of the solution to the volume of the aliquot. The slope of the line mentioned above is altered by the pH of the solution and by the presence of such electrolytes as Na_2SO_4. We have as yet not been able to buffer the solution or to control the other factors. Due to these facts fluoride cannot be estimated by a comparison of the fading caused by an unknown quantity fluoride against a standard, and we have used the method described above as the only one applicable. The method has been found to give good checks even in the presence of 0.1 g. Na_2SO_4, 0.04 g. NaCl, 0.1 g. $NaNO_3$ and 5 cc. saturated silicic acid, and it has been applied successfully to the analysis for fluoride in solutions of NaF prepared from SiF_4 distillates. Further work is in progress in which it is planned to apply the method to the analysis for fluoride in biological material.

5912

Prenatal Growth of Human Testes and Ovaries.

HERBERT WALD AND RICHARD E. SCAMMON.

From the Department of Anatomy, University of Minnesota, and the Department of Anatomy, University of Chicago.

The ponderal growth of the testes, with respect to body weight, has been studied from a series of 89 human fetuses, ranging from 16.3 to 3600 gm. in total body weight.[1] When the weight of the fetal testes is plotted against the weight of the body as a whole, it follows a rectilinear course which may be approximated by the expression:

$$T\,W = 0.000225\,B\,W + 0.0172 \qquad (1)$$

where TW is the testes weight in gm., and BW is the total weight of the body in gm. This formula was computed from the individual values by the method of means. The calculated values thus obtained show a mean absolute deviation of 0.025 gm., and a mean relative deviation of 8.03% from the observed values. This relationship of the weight of the testes to total body weight is similar to that exhibited by the weights of many other organs and parts of the body in the fetal period.

In order to obtain the rate of growth, an age-testes weight formula was obtained by substituting the Scammon-Calkins body weight-age formula[2] in the testes weight-body weight formula equation (1), thus

$$T\,W = 0.225\ (0.561 - 0.366T - 0.061T^2) - 0.0172^{*} \qquad (2)$$

Rate was calculated by obtaining the first derivative. The resulting rectilinear progression in rate is illustrated in Fig. 2.

The relation between testes-weight and body-length, as determined from 89 observations, is shown in Fig. 3. The empirical formula, determined from the averages of testes weight for 5 cm. intervals of body-length from 5 to 55 cm. inclusive (weighted by the square root of the number of observations in each interval) may be expressed as:

$$T\,W = 7.554e^{0.091165BL} \qquad (3)$$

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[1] Material from the autopsy records of the University of Minnseota, and a collation of published records including the extensive data of Borovansky, (L. Borovansky, *Bull. internat. de l'Acad. d. sc. d. Boheme*, 1930, 15).

[2] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 157.

* Constant 0.225 modified for kilograms, coefficient of T corrected from 0.336 tc 0.366. Error due to typographical error in original paper.

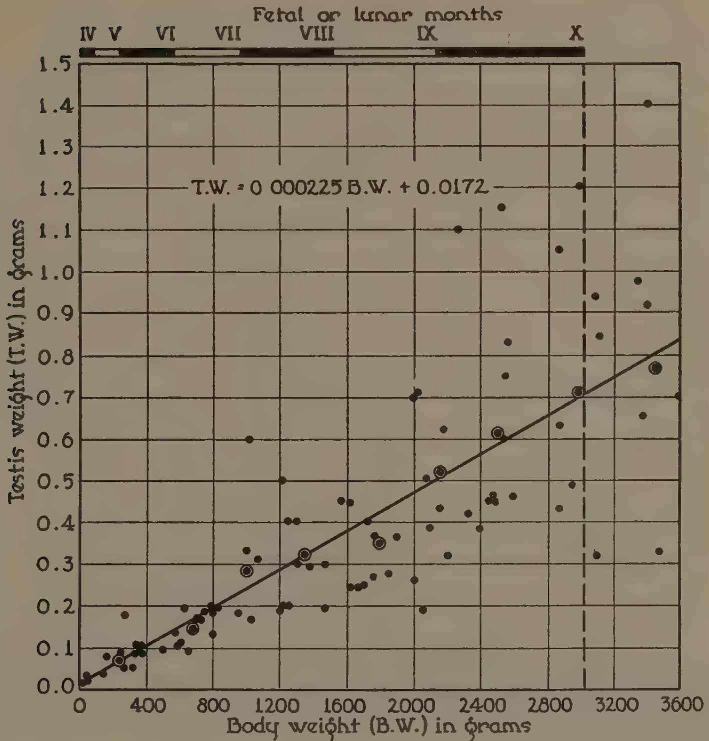

FIG. 1.

Ponderal growth of the human testes with respect to total body weight in the fetal period. The body weight (in gm.) is indicated on the base line of the graph. The estimated age (as computed from the "approximate" formula of Scammon and Calkins) is represented by panels along the upper margin of the figure. The observed individual testes weights are indicated by solid dots. The observed, mean testes weights for 400 gm. intervals of total body-weight are indicated by the circled dots. The graphical expression of the empirical formula for the relation of testes-weight to body-weight is represented by the heavy solid line. The vertical broken line in the field represents the computed birth value of body weight.

where TW is the testes weight in mg., e is the base of the natural logarithms, and BL is the total, or crown-heel body length. The mean, weighted, absolute deviation of the observed from the calculated means as determined by the above formula is 0.048 gm. and the corresponding relative deviation is 13.51%.

This is a different type of exponential curve than that used by Scammon and co-workers to express the relation of the weights of many other organs and body parts to body length in the fetal period. In these instances the weight of the organ could be ap-

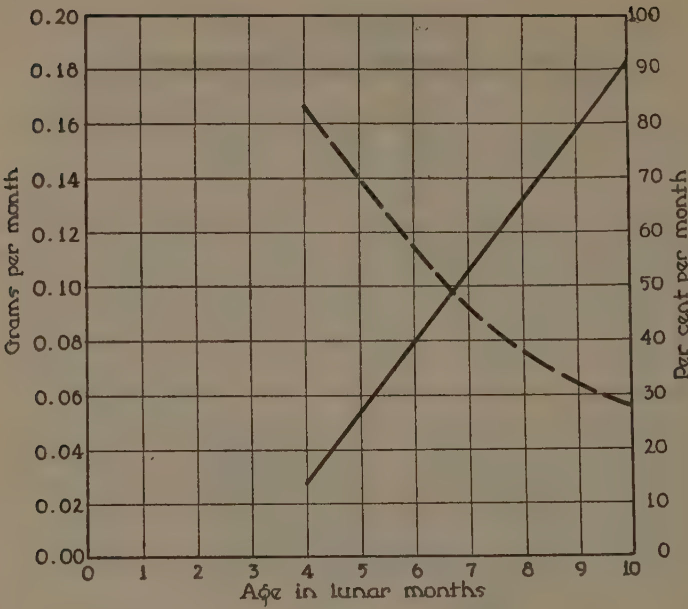

FIG. 2.

The solid line is a semischematic curve illustrating the absolute instantaneous velocity of growth of weight (gm. per lunar month) of the testes in the prenatal period. The broken line is a semidiagrammatic curve illustrating the changes in relative instantaneous velocity (instantaneous velocity ratio) of the testes in the prenatal period.

proximated by a small fraction of the body length raised to a power approaching a cube. This follows the general formula:

$$OW = aBL^b \qquad (4)$$

in which OW represents organ weight and BL, body length.

This difference may be due to the inadequacy of the data, and not to a fundamental difference in the nature of the relationship of increase of testes weight with body-length.

These figures indicate: (a) that the ponderal growth of the testes in the fetal period is quite comparable to that of the body as a whole, and that of most of its major parts and organs; and (b) that although the growth of the absolute weight of the testes is proportional to the growth in body weight during the fetal period, the *relative* weight of the organ, with respect to body weight, undergoes a reduction during this period from 0.143% at 3 lunar months to 0.023% at birth.

TABLE I.

Body Weight-Testes Weight in the Fetal Period.

Intervals of body weight. (gm.)	No. of cases	Mean body weight (gm.)	Testes Weight (gm.)				Deviation		Age* in lunar months
			Observed			Calculated Mean			
			Mean	Maximum	Minimum		(gm.)	%	
0 to 400	13	230.0	0.070	0.180	0.011	0.069	0.001	1.43	4.89
400 to 800	12	674.0	0.141	0.189	0.096	0.169	0.028	19.86	6.29
800 to 1200	7	1010.6	0.286	0.600	0.169	0.245	0.041	14.36	7.05
1200 to 1600	10	1347.0	0.324	0.500	0.194	0.321	0.003	0.93	7.68
1600 to 2000	13	1774.0	0.354	0.720	0.245	0.416	0.062	17.61	8.37
2000 to 2400	9	2164.0	0.520	1.100	0.190	0.504	0.016	3.08	8.94
2400 to 2800	9	2517.0	0.615	1.150	0.391	0.584	0.021	3.41	9.41
2800 to 3200	9	2995.0	0.717	1.200	0.315	0.691	0.028	3.91	9.99
3200 to 3600	7	3453.0	0.766	1.400	0.332	0.794	0.028	3.66	10.51

*Age calculated with Scammon-Calkins age-body weight formula.

TABLE II.

Body Length-Testes Weight in Fetal Period.

Body length intervals (cm.)	No. of cases	Mean body length (cm.)	Testes Weight (mg.)				Deviation	
			Observed			Calculated Mean		
			Mean	Minimum	Maximum		(mg.)	%
5 to 10	1	9.8	11.0	11	11	18.4	7.4	67.3
10 to 15	2	11.2	15.0	15	15	20.9	5.9	39.3
15 to 20	1	19.1	37.0	37	37	43.0	6.0	16.2
20 to 25	6	23.4	98.3	50	180	63.7	34.6	35.2
25 to 30	5	27.3	104.4	50	189	90.9	13.5	12.9
30 to 35	7	32.1	128.9	93	166	140.8	11.9	9.2
35 to 40	16	37.4	281.4	130	600	229.1	52.3	18.6
40 to 45	17	42.6	366.2	188	720	277.5	11.3	3.1
45 to 50	19	47.1	531.6	257	1150	553.2	21.6	4.1
50 to 55	15	52.9	793.3	332	1400	937.7	144.4	18.2

TABLE III.

Weight of Ovaries in the Fetal Period.

Body length intervals (cm.)	No. of cases	Mean body length (cm.)	Weight of Ovaries (gm.)		
			Mean	Minimum	Maximum
10 to 15	1	11.8	0.0130	0.013	0.013
15 to 20	3	17.9	0.0387	0.034	0.045
20 to 25	2	24.0	0.0495	0.042	0.057
25 to 30	9	28.6	0.1344	0.067	0.248
30 to 35	9	33.5	0.1662	0.040	0.381
35 to 40	13	38.0	0.1645	0.040	0.332
40 to 45	10	42.7	0.2607	0.198	0.400
45 to 50	18	47.6	0.2005	0.080	0.420
50 to 55	14	51.8	0.2436	0.090	0.586

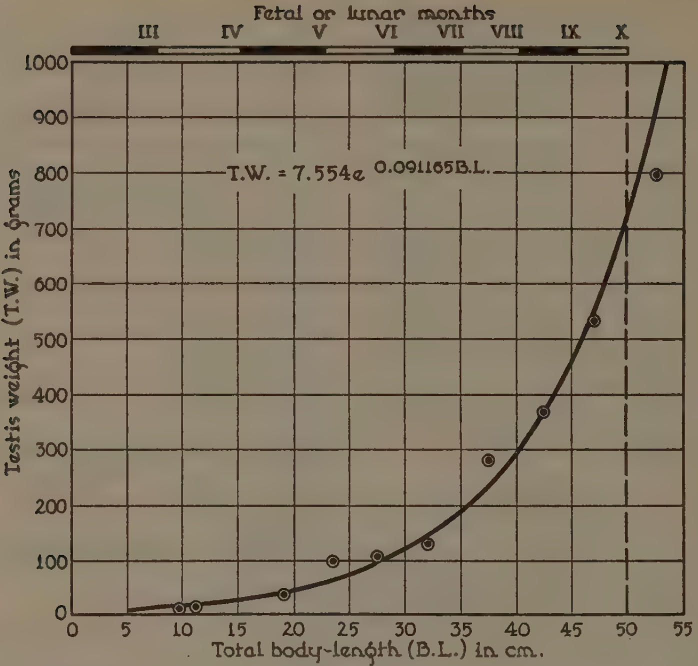

FIG. 3.

A graph illustrating the ponderal growth of the testes with respect to crown-heel body-length. The body length (in cm.) is indicated on the base line of the graph. The estimated age (as computed from the empirical formula of Scammon and Calkins) is represented by the panel along the upper margin of the figure. The observed mean testes weight for 5 cm. intervals of total body length are indicated by circled dots. The vertical broken line in the field is the computed birth value of body length.

An attempt was made to analyze a series of 79 cases of ovary weights. However, due to the great variability in this material, no reasonable trend line could be obtained.

5913

Histologic Study of the Intestine in Simple Obstruction.*

HERBERT A. CARLSON AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

Definite circulatory, inflammatory and degenerative changes occurring in the bowel wall in simple obstruction have been reported by several observers on the basis of anatomical studies. Most of the material has been obtained from dogs with experimental obstruction. Chenut[1] mentioned vascular congestion, desquamation of epithelium, leucocytic infiltration and decreased staining power of the cells. Hartwell, Hoguet, and Beekman[2] described vascular congestion, round cell or leukocytic infiltration, hemorrhages, edema of submucosa, exfoliation of lining cells and necrosis of the mucosa at certain points. VanBeuren[3] also described marked changes occurring in simple obstruction such as areas of gangrene and vascular thrombosis. Braun and Wortmann[4] on the basis of the work of Hoffman, Reichel, Enderlen, Hotz, and others described edema and venous stasis of the proximal bowel segment and a loosening of the fibrous and muscular layers. As venous stasis increased more marked changes were noted, extravasations of blood, thrombosis of small veins, infiltration of the tissues, and finally ulceration of the mucosa and necrosis of the bowel wall. Gatch[5] states that gangrene may regularly occur in simple obstruction as a result of increased intraluminary tension, except in high (duodenal) or low (colonic) obstructions. Elman and Hartmann[6] found examples of discoloration or gangrene of the bowel in dogs with obstructions of the ileum but concluded that death in low intestinal obstruction is not necessarily due to gangrene or necrosis of the obstructed intestine.

The present histologic study of the obstructed intestine was undertaken in order to determine whether the anatomic changes occurring in the bowel in simple obstruction differ only in degree

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* This work was supported in part by Grant 244 allowed by the Committee on Scientific Research of the American Medical Association.

[1] Chenut, A., *Rev. d. Chir.*, 1926, **64**, 1474.

[2] Hartwell, J. A., Hoguet, J. P., and Beekman, F., *Arch. Int. Med.*, 1914, **13**, 701.

[3] VanBeuren, F. T., Jr., *Ann. Surg.*, 1930, **72**, 610.

[4] Braun, H., and Wortmann, W., *Der Darmverschluss.* Julius Springer, Berlin, 1924, 41.

[5] Gatch, W. D., *Ill. Med. J.*, 1931, **60**, 236.

[6] Elman, R., and Hartmann, A. F., *Surg. Gynec. and Ob.*, 1931, **53**, 307.

from those occurring in strangulation obstruction and whether circulatory and degenerative changes can regularly be demonstrated in the bowel in the presence of simple obstruction.

Method. In the experiments from which the material was obtained, blockage of the intestine was produced in dogs by dividing the bowel, ligating the 2 ends and inverting them with purse-string sutures. The animals were sacrificed when they appeared moribund and specimens of the intestine removed for microscopic examination. A few of the dogs died unexpectedly and specimens were taken in these at postmortem. Sections showing definite postmortem changes were eliminated from the series. One or 2 specimens were taken from the bowel distal to the obstruction and one to 4 specimens were removed from the obstructed proximal segment at various levels between the pylorus and the point of obstruction. In the earlier cases sections were taken at random but in later experiments the entire bowel was opened and inspected grossly and specimens selected from any areas that appeared to deviate from the normal in gross appearance. The tissues were fixed in either formalin or Zenker's solution, imbedded in paraffin and microscopic slides prepared employing the hematoxylin eosin stain.

The observations are based upon 9 cases of duodenal obstruction (3 to 6 days' duration); 17 of ileal obstruction (2 to 14 days' duration), and 9 of colonic obstruction (2 to 19 days' duration).

Results. The gross appearance of the bowel was of some value in elaboration of the microscopic picture. A redness of the distended obstructed loop was observed almost uniformly. This rubor undoubtedly means congestion and it is our experience that the gross appearance is a more reliable criterion of congestion than the microscopic.

In duodenal obstruction microscopic changes were rarely observed. Edema and congestion of the mucosa and submucosa which are difficult to evaluate on microscopic examination and shortening of the villi occurred, but none of these findings were constant. No interstitial hemorrhages or areas of necrosis were observed.

In the case of ileal obstruction greater changes were noted, probably because some of these animals survived for a longer period of time. But even in this group in many of the sections no distinct difference could be demonstrated between sections taken above and below the obstruction. Edema of the mucosa and shortening of the villi were often observed. A clubbed appearance of the villi was a prominent feature in some of the sections. Congestion of the bowel wall was sometimes noted but was not of severe degree, and in no instances were any intramural hemorrhages encountered. Leucocytic infiltration of the bowel wall occurred in one animal dying of peritonitis. An ulcer of the duodenum was found in one of the dogs in this series.

Microscopic sections of the colon as late as 19 days after the production of an obstruction of the colon failed to reveal changes other than an ironing out of the plicae and in some instances a slight congestion.

Comment. The findings reported here fail to confirm some of the previous studies on the pathology of simple intestinal obstruction. The loss of epithelium particularly on the tips of villi was noted occasionally both above and below the obstruction and was evidently due in part at least to mechanical removal of epithelium by the microtome knife during preparation of the section. It may occur more commonly in the obstructed loop because of edema of the mucosa. We find no evidence that loss of epithelium is a constant or characteristic feature of the pathology of simple intestinal obstruction.

The marked circulatory changes and necrosis referred to by other authors were not observed in our specimens. The only instance of ulceration was a duodenal ulcer in an animal with an obstructed ileum. The occurrence of such an ulcer is of doubtful significance.

Necrosis of the proximal bowel wall was not encountered in this series of 35 dogs. That it may occur following the establishment of simple obstruction in the colon due to the presence of a competent ileo-caecal valve is not to be denied. Its clinical occurrence is well known in obstructions of the colon and especially due to carcinoma at the sigmoid flexure, the necrosis with ensuant gangrene or perforation being almost invariably found in the cecum, the most distensible portion of the bowel.

Evidence for the presence of vascular disturbances in simple obstruction is to be found in the studies of Van Zwallenburg,[7] Gatch, Trusler and Ayers,[8] and Dragstedt, Long and Millet,[9] which demonstrate stagnation or even complete cessation of the intramural intestinal circulation in loops of bowel artificially subjected to distention. The pressures employed were usually from 30 to 200 mm. of mercury.

However, hollow muscular viscera, such as the bladder, stomach, and intestine, exhibit great ability to accommodate themselves to altered capacities without significant changes in tension, and it would appear that the intra-intestinal pressures necessary to arrest the blood flow in the wall of the bowel are in excess of pressures observed in the bowel during the course of obstruction. As a matter of fact, Owings, McIntosh, Stone and Weinberg[10] found in simple obstruction of the small intestine in dogs that the sustained intra-intestinal pressure was only 6 to 8 cm. of water (about twice the normal) although under the influences of vigorous peristalsis the pressure at times reached a maximum of 30 to 60 cm. of water.

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[7] Van Zwallenburg, C., *Ann. Surg.*, 1907, **46**, 780.

[8] Gatch, W. D., Trusler, H. M., and Ayers, K. D., *Arch. Surg.*, 1927, **14**, 1215.

[9] Dragstedt, C. A., Lang, V. F., and Millet, R. F., *Arch. Surg.*, 1929, **18**, 2257.

Further evidence that the circulatory changes usually are not extreme is afforded by the experiments of Morton,[11] who injected the intestinal vessels with India ink and found that although the vessels of the obstructed segment were dilated, the capillary loops had intact walls.

Conclusions. The material presented here leads to the conclusion that in simple obstruction only slight changes (congestion, edema) can be demonstrated and frequently no alteration of microscopic structure is to be observed. Grossly the bowel regularly appears hyperemic but no inflammatory or degenerative changes are observed on histologic study.

5914

Length of Life Following Various Types of Strangulation Obstruction in Dogs.*

HORACE G. SCOTT AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

In a series of 12 dogs, Foster and Hausler[1] reported the survival period following strangulation obstruction to be less than 30 hours. The lengths of intestine strangulated in their series varied between 5 and 26 inches. They tabulated their results and were able to show that the length of life was inversely related to the length of bowel strangulated. In an earlier paper by Murphy and Vincent[2] the survival period in a series of 12 cats was reported. These authors varied the type of strangulation which they produced but not the length of gut. Their tabulated results show that the length of life depends upon the type of strangulation produced. Their animals in which the veins were occluded and the arteries left intact died much more rapidly than the animals in which the arteries were completely shut off.

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[10] Owings, J. C., McIntosh, C. A., Stone, H. B., and Weinberg, J. A., *Ann. Surg.*, 1928, **17**, 507.

[11] Morton, J. J., *Arch. Surg.*, 1929, **18**, 1119.

* This work was supported in part by Grant 244 allowed by the Committee on Scientific Research of the American Medical Association.

[1] Foster, W. C., and Hausler, R. W., *Arch. Int. Med.*, 1924, **34**, 697.

[2] Murphy, Fred T., and Vincent, Beth., *Boston Med. and Surg. J.*, 1911, **165**, 684.

A series of similar experiments were carried out in this department in an attempt to confirm the work of these authors and determine if possible the rôle that the arteries and veins play individually and collectively in the survival period. To evaluate the rôle of each, our experiments were divided into 4 groups.

Methods. In group number I no attempt was made to control the relative degree of arterial and venous occlusion. We hoped by this method to obtain some idea of the variations in length of life which one might encounter when a constricting band was passed about the mesenteric pedicle and bowel wall. Binding tape was used for this purpose. The tape, 1 cm. in width, was passed about the mesenteric vessels and then carried around the bowel wall and tied. The lumen of the bowel as well as its blood supply was thus occluded. It is evident that the degree of occlusion of the arteries and veins in this group depends upon the tightness of the encircling tape. In the succeeding 3 groups, binding tape was used merely to occlude the lumen of the bowel at the ends of the segment strangulated. The mesenteric vessels were occluded by ligating and severing them individually. In group II, all of the arteries and veins to the loop selected were ligated and severed. In group III, all the arteries were ligated, the veins being left intact. In group IV, only the veins were ligated, the arteries being left intact. The operative procedures were carried out under ether morphine anesthesia and aseptic technique was employed in every case. Fifty-six experiments form the basis for this report.

Results. In group I in which an encirclement ligature was placed about the mesentery and bowel in 14 dogs, the survival period varied between 4 to 28 hours. The mean length of life for the group was 16 hours. (Table I.) The length of bowel strangulated in this group varied between 1 and 5 feet. Although the dog with the shortest obstruction lived the longest, we find that the dog with the longest obstruction lived longer than a number of other dogs with shorter obstructions. These apparent exceptions to Foster and Hausler's conclusions were anticipated in view of the fact that the relative degree of arterial and venous obstruction could not be ac-

TABLE I.

Encirclement Ligature.

Exp. No.	Length of Gut in Feet	Length of Life in Hours
46	1	28
45	2½	18
49	3	16
38	3	12½
33	3	4
47	3	7
40	3½	18
41	4	8½
30	4	12
42	4	19
44	4	16½
48	4	15
39	4½	18
43	5	18
		Av. 16 hours

TABLE II.

Ligation of Arteries and Veins.

Exp. No.	Length of Gut in Feet	Length of Life in Hours
46	1	27
70	1	20
72	1	15
90	1	32
64	2	18
67	2	21
81	2	20
83	2	17
84	2	18
89	2	30
45	3	18
71	3	20
82	3	17
49	4	20
65	4	16
79	4	12
48	5	15
69	5	11
		Av. 19 hours

TABLE III.

Ligation of Arteries.

Exp. No.	Length of Gut in Feet	Length of Life in Hours
94	1	18
96	2	24
97	2	19
116	2	20
115	2½	18½
85	3	20
87	3	24
88	3	22
98	4	15
		Av. 20 hours

TABLE IV

Ligation of Veins.

Exp. No.	Length of Gut in Feet	Length of Life in Hours
76	3	4
73	3	9
68	3	8½
62	3	4¼
61	3	11½
52	3½	3½
57	4	4
60	5	6½
59	5	5
58	5	12
56	5	4
55	5	4
53	4½	3½
54	4½	6¼
51	5½	2½
		Av. 5½ hrs.

curately controlled. Further, the weights of the animals varied between 10 and 25 kilos. Therefore, the actual length of gut obstructed gives one no indication of the relative amount obstructed, which is really of greater importance. In group II, in which the arteries and veins were completely ligated in 18 dogs, the shortest survival period was 11 hours and the longest 32. The length of gut strangulated varied as in the preceding group between 1 and 5 feet. The mean length of life for this group was 19 hours. In group III, in which the arteries were tied in 9 dogs, the shortest survival period was 15 hours and the longest 24. The mean length of life was 20 hours. The length of bowel strangulated varied between 1 and 4 feet. In group IV, in which the veins were tied in 15 cases, the shortest survival period was 2½ hours and the longest 11½ hours. The mean length of life was 5½ hours. The gut length varied between 3 and 5½ feet.

Comment. Analyzing these statistics, one finds that the average length of life in the venous obstruction group is approximately ¼ that of the arterial group, and the combined artery and vein ligature group; it is approximately ⅓ that of group I or the encirclement group in which the degree of arterial occlusion was not absolute in all cases. At autopsy, there was a marked difference in the appearance of the strangulated bowels in the various groups. In group IV, the bowel was always markedly distended; its color was practically always a deep dark mahogany; the serosa was lusterless and not infrequently had a fibrinous exudate on its surface. The serosa was split in a few cases but there was no perforation of the bowel in any dog of this group. On opening the bowel, it was found to contain a large amount of dark bloody fluid of high hemoglobin content. The wall of bowel was always thickened. In some cases, it was as much as 4 times its normal weight. There were frank diffuse hemorrhages throughout the mesentery, being most marked along the course of the mesenteric vessels. The peritoneal cavity of these dogs usually contained from 50 to 250 cc. of a hemorrhagic fluid of rather low hemoglobin content.

The findings at autopsy in the other groups were quite different from these, except for those in group I that died rather early. These dogs usually presented similar findings. In the artery and vein ligature groups (group II), and the arterial ligature group (group III), the bowel was usually not distended, neither was it covered with fibrin. The color in a few cases was grayish yellow (anemic) but the majority of the segments had undergone hemorrhagic necrosis. The wall was of a jelly-like consistency, of a mahogany color, usually thinner than normal and quite frequently perforated in one or more places. The peritoneal cavity usually contained from 100 to 500 cc. of a dark bloody, foul smelling fluid of a high total protein content, in marked contrast to the odorless fluid in the venous strangulation group.

Conclusion. Although the length of bowel involved is of importance in shortening the survival period following strangulation obstructions, the type of obstruction is of greater significance.

5915

Blood Pressure Changes Correlated with Time, Length and Type of Intestinal Strangulation in Dogs.*

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Introduction. A significant fall in blood pressure was observed following release of the constricting ligature in cases of strangulation obstruction reported from this laboratory several years ago.[1] Just prior to that, Foster and Hausler,[2] writing on the same subject, pointed out a direct relationship between the drop in pressure and the length of gut strangulated. Believing that the type of strangulation may vary greatly, depending on the degree of arterial or venous occlusion present, and knowing that the time factor is of great importance, we decided to control these variables in an effort to determine whether or not there was any correlation between the blood pressure on the one hand and the time, length and type of strangulation produced on the other.

Methods. The carotid artery was cannulated in 30 dogs and a tracing made of the blood pressure during the course of various types of vascular occlusions. In each experiment the time as well as the length and site of bowel strangulated were noted. In order to control the degree of arterial and venous occlusion, the experiments were divided into 4 groups, as in the preceding paper on "Length of life following various types of strangulation obstruction." The methods employed were identical.

Results. In group I (encirclement obstruction), the bowel strangulated varied between 1 and 11½ feet. The time factor between 3 and 7 hours. The blood pressure prior to strangulation was recorded in 5 of these experiments, while the resulting pressure after from 3 to 7 hours is known in every case. The lowest pressure recorded after this interval was 70 mm. of Hg. pressure and the highest 140 mm. In the 7 dogs in which the length of gut was less than 3 feet, the blood pressure had not fallen in any case below 100 mm. In the 9 remaining dogs in which the segment strangulated was more than 3 feet in length, the pressure at time of release was less than 100 mm. in 6 cases. The time at which the release

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* This work was supported in part by Grant 244 allowed by the Committee on Scientific Research of the American Medical Association.

[1] Wangensteen, O. H., and Loucks, Milo, *Arch. Surg.*, 1928, **16**, 1089.

[2] Foster, W. C., and Hausler, R. W., *Arch. Int. Med.*, 1924, **34**, 697.

was effected varied in each instance, but the time interval was approximately the same in both divisions of this group with but one exception.

In group II, in which the arteries and veins were ligated the pressure was found to be within normal limits after from 4 to 6 hours.

In group III, in which the arteries were ligated, the pressure was also found to be within normal limits after from 4 to 6 hours. Tracings were then taken after 18½ hours on 2 which appeared moribund. The one showed a mean pressure of 20 mm. and the other 70 mm. The first died ½ hour later and the second 1½ hours later.

In group IV, in which the veins were ligated in 10 dogs, the pressure was below 70 mm. in every instance after from 1 to 5 hours. Three of the 10 animals were dead. One animal in which the superior mesenteric vein was ligated died within one hour. Two of 3 dogs in which the veins to a 5½ foot segment were ligated, died within 4 hours with consistent decline in blood pressure during

TABLE I.
Encirclement Strangulation. (Ligation of bowel and mesentery.)

Exp. No.	Dog Wt.	Gut Length	Strang. Time	Initial Press.	Resulting Press.
	kilos	ft.	hours	mm.	mm.
5	20	1	4		140
14	5	1	5		140
1	11.3	1	3		100
2	16	1	4½		130
3	9	1	5		100
8	11	3	4¼		130
7	20	3	4		110
47	8	4	5	110	90
9	19	5	4½	130	100
11	10	5	3¾		94
12	8	4	4½		110
32		4	5	100	90
13	10	5	4		90
10	5.5	5	4½		80
31		7	7	140	120
36	25.5	11½	4½	110	70

TABLE II.
Ligation of Arteries and Veins.

Exp. No.	Dog Wt.	Gut Length	Strang. Time	Resulting Press.
	kilos	ft.	hours	mm.
17	20	4½	4¾	120
16	14	5	4	145
16	14	5	6	135

the course of the experiments. One animal in which the veins to a 5-foot segment were occluded, was still alive at the end of 4 hours, but had a blood pressure of only 54 mm. The remaining 6 dogs in which the segment was less than 3 feet were all alive after an interval varying from 3 to 5½ hours.

TABLE III.
Ligation of Arteries.

Exp. No.	Dog Wt.	Gut Length	Strang. Time	Resulting Press.
	kilos	ft.	hours	mm.
115	18	2½	18½	20
115	18	2½	19	0
116	22	2	18½	70
116	22	2	20	0

TABLE IV.
Ligation of Veins.

Exp. No.	Dog Wt.	Gut Length	Strang. Time	Initial Press.	Resulting Press.
	kilos	ft.	hours	mm.	mm.
101	13.5	2	2	120	56
100	20	2½	4	150	60
93	9	2½	4½	120	60
99	19	2½	5½	140	70
91	21	2½	5	140	70
92	12	3	3	110	38
55	20	5	4	140	0
56	16.2	5	4	150	56
51	13	5½	2½	120	0
114	10	S. Mes. V.	1	140	0

Comment. The marked early fall in blood pressure which is seen in the group in which the veins are ligated points strongly to death resulting from a loss of blood from the general circulation. Blalock,[3] working on the etiology of traumatic shock has come to a similar conclusion, namely, that a loss of blood into the traumatized tissues occurs in sufficient amounts to account for the observed fall in blood pressure.

Conclusions. There appears to be a direct correlation between the fall in blood pressure and the time, length, and type of vascular occlusions. In general, the longer the time and the longer the loop, the greater the fall in blood pressure. The type of obstruction, however, is of even greater importance than the time and length of the occlusion. In those instances in which the veins were ligated and in the encirclement strangulations (Group I), in which the arteries were not entirely occluded, the fall in pressure was much more rapid than in those instances (Group III), in which the arteries were absolutely occluded regardless of the condition of the veins.

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[3] Blalock, Alfred, *Arch. Surg.*, 1930, **20**, 959.

5916

Liver Autolysis in the Peritoneal Cavity of the Dog.

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Mann observed in his experiments with hepatectomy in dogs that when small pieces of liver tissue were left behind in the peritoneal cavity, the dogs invariably died of a severe peritonitis in less than 24 hours. Mason[1] and coworkers confirmed Mann's observations, studied the blood chemistry in such dogs, and found that saline extracts of autolized dog's liver injected intravenously proved very toxic. If the liver was *not* autolized, no toxic reactions were observed. They described the typical picture of dogs dying following deposition of pieces of dog's liver into the peritoneal cavity. One to 300 cc. of a serosanguinous fluid was usually present and the peritoneal surfaces were reddened. After 24 hours, liver placed into the cavity could hardly be identified except as a friable mushy mass which contained gas. Wangensteen[2] showed the relative non-toxicity of rat's liver and adult dog's kidney compared to adult dog's liver. He also showed the existence of a quantitative tolerance of dog's liver introduced into the rat's peritoneal cavity, the rats usually not dying till 15 gm. of dog's liver per kilo body weight of rat had been exceeded. Ellis and Dragstedt[3] indicated that an anaerobic gas forming bacillus commonly found in the dog's liver was responsible for death in liver autolysis *in vivo*. They identified this organism closely with the Welch bacillus. Later Rewbridge[5] showed that introduction of sterile bile salts into the peritoneal cavity of dogs caused a peritonitis death similar to that of liver autolysis. Still later, Andrews[6] contended that the cause of death in liver autolysis was primarily due to a toxic agent extractable from fresh normal adult dog's liver and independent of bacterial activity. However, he claimed such deaths were accompanied by presence of Welch-like organisms in the peritoneal cavity at death.

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[1] Mason, E. C., Davidson, C. C., *et al.*, *J. Lab. and Clin. Med.*, 1924, **10**, 622, 906, 977.

[2] Wangensteen, O. H., *Endokrinol.*, 1928, **2**, 170.

[3] Ellis, J. C., and Dragstedt, L. R., *Arch. Surg.*, 1930, **20**, 8.

[4] Andrews, A., and Hrdina, L. S., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 987.

[5] Rewbridge, A. G., *Surg. Gynec. and Obstet.*, 1931, **52**, 205.

The purpose of this paper is to repeat some of the work already done on the subject and attempt to determine more conclusively by the employment of filtrates of fresh liver tissue and liver tissue treated in various ways, as well as filtrates of cultures of the organisms found in liver tissue, whether the bacteria and their toxin are solely responsible for the death of the animal or whether other toxic products peculiar to liver tissue itself as contended by Andrews also plays a significant rôle in the death of the animal.

Method. Experimental samples of dog's liver, filtrates and ex-

TABLE I.

Substance Introduced.		No. Dogs	Died	Survived
1	Rat liver	2	0	2
2	Adult dog kidney	1	0	1
3	” ” liver—ground	4	4	0
4	Foetal dog liver—ground	1	0	1
5	Adult dog liver—ground—autoclaved (see Exp. 18 for similar result)	1	0	1
6	Adult dog liver—ground—boiled ½ minute	2	0	2
	” ” 3 ”	2	0	2
	” ” 3 ” —incubated	1	1	0
7	Broth culture dog liver anaerobes— 4 cc., 6 cc., 30 cc.	3	0	3
	—40 cc., 60 cc., 100 cc.	3	3	0
8	” ” ” ” ” —filtrate	1	1	0
9	” ” ” ” ” ” —boiled	1	0	1
10	” media control—filtrate	3	0	3
11	” culture dog liver anaerobe—autoclaved liver—ground			
	2.5 cc. of culture— ” ” ”	1	1	0
	15.0 cc. ” ” — ” ” ”	1	1	0
12	Adult dog liver—ground—Aqueous extract—extract unfiltered	1	0	1
13	Adult dog liver—ground—Aqueous extract—extract filtered	4	0	4
14	Adult dog liver—ground—incubated—Aqueous extract—extract filtered—boiled	1	0	1
15	Adult dog liver—ground—incubated—Aqueous extract—extract unfiltered	1	1	0
16	Adult dog liver—ground—incubated—Aqueous extract—extract filtered	3	2	1
17	Foetal dog liver—ground—incubated—Aqueous extract—extract filtered	2	0	2
18	Adult dog liver—ground—autoclaved—plus filtrate fresh adult dog liver, combination incubated	3	0	3
19	Adult dog liver—ground—ether extract	2	0	2
20	” ” ” ” —alcohol extract	4	0	4
21	Peritoneal fluid of dogs dying from liver autolysis—filtrate	2	0	2

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[6] Andrews, A., Rewbridge, W. G., and Hrdina, L., *Surg. Gynec. and Obstet.*, 1931, **53**, 176.

tracts of such liver, and cultures of anerobic organisms were introduced into the peritoneal cavities of adult dogs. The samples of liver treated in the various ways described in Table I were introduced in amounts always equal to 4 gm. per kilo dog body weight. The filtrates and extracts were introduced in amounts equivalent to corresponding amounts of liver, namely 4 gm. per kilo. The amounts of bacterial culture introduced are given in the table. All filtrates made were Berkefeld filtrates. All extracts were either with physiologic saline, ether, or alcohol as specified. All bacterial cultures were approximately 18 hours old and were composed of gas forming anaerobic gram positive bacilli isolated from adult dog's liver. These organisms were used in pure form and were beyond question the same as those used by other workers in this field. All dogs reported having died in these experiments, died inside of 36 hours. Those that did not die lived several weeks when used for other purposes.

Results. Experiments 1, 2, and 4. As noted in the table, ground rat liver, foetal dog liver and adult dog kidney did not kill. These tissues were all cultured and found bacteriologically sterile.

Experiment 3. Ground adult dog liver invariably killed in the amounts specified. It always contained gas forming anaerobes.

Experiment 5. Autoclaved ground adult dog liver never killed. It was always sterile.

Experiment 6. Boiling ground fresh adult dog's liver probably kills vegetative forms but not spores of the anaerobes and therefore did not kill. Incubating the boiled liver perhaps liberates vegetative forms and therefore kills.

Experiment 7. At least 40 cc. suspension of the anaerobes alone was found necessary here to kill.

Experiments 8 and 9. Filtrate of such a suspension kills but not after being boiled, indicating presence of a thermolabile toxin.

Experiment 10. Filtrates of liver peptone broth similar to that in which the organisms were grown were introduced intraperitoneally as controls. They did not kill.

Experiment 11. Sublethal amounts of bacterial suspension plus autoclaved ground liver kills.

Experiments 12 and 13. Aqueous extracts of fresh ground adult dog's liver did not kill. It contained no bacterial toxin.

Experiments 14, 15, and 16. When the liver was first incubated, such extracts did kill, but again, not after boiling. This would point towards presence of a toxin produced by bacteria and destroyed by heat.

Experiment 17. Filtrates of incubated foetal liver did not kill. No bacteria were present in this liver in the beginning.

Experiment 18. When autoclaved ground adult dog liver plus filtrates of fresh ground adult dog liver was allowed to incubate the animals survived. This would show that water soluble enzymes from the fresh ground liver acting on autoclaved liver unaided by bacteria produced no toxic substance. This would point against the presence of a toxin produced by aseptic enzyme autolysis of liver.

Experiments 19 and 20. Ether and alcohol extracts of ground fresh adult dog's liver did not kill.

Experiment 21. Filtrates of peritoneal fluid from dogs dying of liver autolysis proved non-toxic as already shown by others. The toxins are already probably absorbed by the host.

Bile salts are not found in the amounts of dog liver used in these experiments in sufficient quantity to cause death.

Conclusions. 1. Anaerobic bacteria or their toxic products cause death in autolysis of dog's liver *in vivo*. 2. No toxic product was found in the liver responsible for death which was inherent to liver tissue and independent of bacterial activity.

New York Section.

New York Academy of Medicine, January 20, 1932.

5917

Studies of Yellow Fever Virus in Tissue Culture.

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The virus of yellow fever, like most invisible agents of infectious diseases, has not been cultivated on any of the usual bacteriological media. After it had been established that this virus belongs to the group of filterable infectious agents, it seemed worth while to attempt its cultivation *in vitro* by methods successfully developed for other filterable viruses. A strain of yellow fever virus highly pathogenic for mice made it possible for the infectivity of cultures to be tested repeatedly and accurately on a large scale.

Of the several methods of growing viruses in tissue cultures, the use of Carrel dishes has given the best results. Only after repeated attempts were we finally able to carry the virus through the first subculture. The culture medium chiefly used has been a mixture of normal monkey serum and Tyrode solution in which were suspended small fragments of fresh tissue. Many kinds of tissue were used, principally minced kidneys and testicles of guinea pigs and rabbits or minced chicken embryos 8 to 10 days old. The minced embryos have been used almost entirely in the more recent experiments because the virus appeared to remain more uniformly virulent in the cultures containing these tissues.

The virus for the primary culture was prepared by grinding infectious mouse brain in a mortar with 9 times its weight of Tyrode solution and centrifuging the suspension for about 10 minutes at 3000 revolutions per minute. One part of the supernatant fluid was added to 4 parts of the culture medium, and the mixture was distributed in several Carrel dishes, about 2.0 cc. in each. Taking the concentration of infected mouse-brain tissue as one, the final dilution of virus would be 1:50. The cultures and subcultures were always tested for bacteriological sterility. Neither on bacteriological culture medium nor in smears or sections of the tissue cultures was any visible organism apparent. Three to 4 days is the most favorable time between transfers. Subcultures were made in the following manner: The fluid portion of the previous culture was allowed to stand in conical centrifuge tubes to permit as much as possible of the old autolysed tissue to settle. Of the supernatant fluid 0.5 cc. was added to 2.0 cc. of freshly prepared medium in each of several Carrel dishes. The dilution factor of the virus in each subculture, in relation to the virus content of the previous culture, was therefore 5. It is estimated that in the 10th subculture the original virus was diluted about one-half of a billion (5×10^8) times, in the 20th subculture about 5 million billion (5×10^{15}) times. The fact that the last passages were just as infectious as the original culture shows not only that the virus had been preserved but that in all probability multiplication had taken place. The incubation temperature used for the cultivation was 37.5°C.

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The infectivity tests were always done in mice. All mice inoculated intracerebrally with the 22nd subculture showed typical symptoms of encephalitis with paralysis. Sections of brain from these mice revealed characteristic perivascular round cell infiltrations and acidophilic changes in the nuclei of the ganglion cells.

From these experiments it appears that the virus of yellow fever like other filterable viruses needs living cells for growth *in vitro*. This virus is extremely labile and is changed or destroyed by the most minute and undetermined changes in the culture medium.

It is therefore possible to grow yellow fever virus indefinitely by a method which has been used successfully for the continuous cultivation of other viruses.

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[1] Stokes, A., Bauer, J. H., and Hudson, N. P., *Am. J. Trop. Med.*, 1928, **8**, 103.

[2] Theiler, M., *Ann. Trop. Med. and Parasit.*, 1930, **24**, 249.

[3] Haagen, E., *Arch. Exp. Zellforsch.*, 1929, **8**, 499, and **12**, in press.

5918

Lethal Action in the Short-Tailed Mutation in the House Mouse.

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From the Department of Zoology, Columbia University.

Three dominant factors in which the homozygous mutant form does not exist are known in mice, *viz.*, yellow coat color (A^y), black-eyed white (W), and brachyury. In all of these cases crosses *inter se* yield an F_1 of 2 mutants to one normal. Crosses to normals give a 1:1 ratio. These facts are explained by the supposition that the presence of 2 factors for the mutant character has a lethal action at some period during the development. Little and Castle,[1] following Cuénot, first advanced this hypothesis as to yellow mice. Subsequent investigation seems to show that the action takes place very early in embryogeny. Aberle[2] traced the lethal action in black-eyed white mice to an anemic condition. Here the homozygous anemics may survive to birth and live a short time afterwards. The breeding experiments of N. Dobrovalskia-Zavadskia[3] have indicated that a lethal action probably accompanies dominant short-tailedness (brachyury). The length of the tail varies. A few are indistinguishable from normal except for a slight bluntness. Others have no external tail. The latter have a varying number of malformed caudals and a derangement of the sacral and lumbar vertebrae. One or more rigid bends caused by an ankylosis of vertebrae often accompany the shortening. A paralysis of the posterior limbs is occasionally encountered. It seems probable that this latter condition is the result of abnormalities in the sacral and lumbar vertebrae.

My object was to discover the stage at which the lethal action of this gene occurs and to describe its effect on development. In matings of brachyurie × brachyurie embryos are found at the last of the eighth day, while still in the stage of the primary flexure, which show definite abnormalities. From the ratio in which these appear there is no doubt that they represent the missing homozygous type. In the mid-region of the embryo the neural tube, instead of being straight as in the normal condition, is bent from side to side. The ectodermal outer layer of the embryo is thrown into a varying number of blebs or vesicles immediately lateral to the neural tube.

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[1] Little and Castle, *Science*, N. S., 1910, **32**, 868.

[2] Aberle, *Am. J. Anat.*, 1927, **40**, 219.

[3] Dobrovalskia-Zavadskia, N., *C. R. Soc. Biol.*, 1927, **97**; 1928, **98**; 1930, **104**; Dobrovalskia-Zavadskia and Kobozieff, *C. R. Ac. d. Sci.*, 1930, **191**, 352.

These give the impression of the puffing out of the tissue layer by the pressure of internal fluids. The complete or partial absence of somites is a third anomalous condition. Although it is uncertain at present whether or not there are vestiges of somites, certainly no regular segmentation of the body similar to that in normal embryos is found.

Homozygous embryos taken from the uterus early in the tenth day show a very marked contrast to their normal litter mates. At this time the normal has about 30 somites and both anterior and posterior limb buds, the latter just perceptible. In both types the heart beats regularly. The homozygote has no somites and the neural tube is bent as in younger embryos. No trace of the dorsal ectodermal blebs is discernible. Other gross deformities are present. The posterior end of the embryo ends abruptly slightly posterior to the anterior limb buds. In normals the tail tapers and turns spirally. The anterior limb buds are the same size as in normals but are distinctly more dorsal in their origin. There are no posterior buds, although in one case an individual with a single median posterior appendage was found. The size of embryos is considerably below that of normal litter mates. This may be interpreted as an indication of approaching death.

Death takes place during the third quarter of the tenth day. The heart does not beat in homozygous embryos taken from the uterus at this time but can sometimes be slightly stimulated by a needle, indicating that the lethal point is very close to this age. Females examined on the eleventh day show embryos already well started toward resorption. Later stages show resorption as in cases of prenatal mortality from other causes.

The ratio of homozygotes to normal embryos for the litters taken from the uterus on the eighth, ninth, and tenth days agrees closely with the expected 1:3. Out of 256 embryos in 29 litters 60 were of the abnormal type designated as homozygous.

Other defects appearing in serial sections of the embryos have not yet been fully studied, nor have the progressive changes in the gross abnormalities. The earliest manifestation of a difference between the homozygote and the normal must be sought in the seventh and eighth days.

5919

General Hyperthermia with Heat Localization by Radiothermy.

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(Introduced by B. N. Berg.)

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When some heat producing procedure is applied to a localized region of the body, it is difficult to secure any substantial elevation of temperature in that region above that of the body temperature due to the efficient heat regulatory mechanism which the body possesses. The heat produced locally is rapidly dispersed, the other regions of the body actively entering into the process of heat dissipation.[1] In the live anesthetized dog placed in the field of a high frequency current, the different organs heat up at approximately the same rate, the blood serving as a very efficient distributing mechanism of the generated heat.[2] In the following communication it is shown that when the systemic temperature is elevated by radiothermy an increased local temperature may be produced and maintained simultaneously in the vagina by applying additional electrical energy in that area. This mechanism has been employed therapeutically by us in the treatment of pelvic inflammations.

The technique for the systemic elevation of temperature in the human subject by means of the high frequency electric field in the radiotherm has been described by Carpenter and Page.[3] The method consists essentially in placing the subject between the plates of a large condenser which forms part of an oscillating circuit. The oscillations in this circuit are produced by the use of radio tubes of appropriate construction (500 watt screen grid in push pull) with the necessary associated equipment for the attainment of a high frequency electric field of high intensity. The frequency commonly used for this work is approximately 10 million cycles per second, corresponding to a wave length of 30 meters. The condenser plates between which the subject is placed are large enough (50x80 cm.) to produce a field over a reasonable part of the volume of the subject, and are separated from one another by a distance of approximately one meter. The subject rests upon a stretcher between these plates but not in contact with them, and is kept covered during the treatment so as to prevent excessive loss of heat. With a total DC plate current of 0.5 amperes using two UV861 tubes it is possible to produce an elevation of body temperature of about 4°C. in the course of an hour and one-half.

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[1] Binger, C. A. L., and Christie, R. V., Proc. Soc. Exp. Biol. and Med., 1927, **24**, 677; *J. Exp. Med.*, 1927, **46**, 571.

[2] Mortimer, B., *Radiology*, 1931, **16**, 705.

[3] Carpenter, C. M., and Page, A. B., *Science*, 1930, **71**, 450.

In order to produce a simultaneous increase of body temperature with a still higher increase in the temperature of the vagina the following method was employed. With the subject in place between the condenser plates of the radiotherm, an electrode is placed in the vagina. This electrode is connected, through an ammeter, to a small auxiliary metal plate suspended near one of the large condenser plates of the radiotherm. Under these conditions the electrical field produced in the region between the electrode and the condenser plate opposite to the one near which the auxiliary plate is suspended, is considerably higher than it is at any other part of the body. When the region to be heated is located asymetrically in the body of the patient, the pick-up plate is brought near the condenser plate opposite to the affected side. If the region is centrally located in the body the pick-up plate is shifted from one side to the other. With a pick-up plate of about 300 sq. cm. area, the distance between the pick-up and the condenser plate varies from 10 to 30 cm. The readings on the radio-frequency ammeter usually vary from about one to 2 amperes. The localization may also be accomplished in regions where there is no convenient cavity by the use of surface electrodes.

By these means the average result in 50 instances was an elevation of the temperature in the vagina to 42.5°C. during an average time period of 53 minutes. This temperature was determined by a mercury thermometer inserted in the vaginal electrode. Coincidently, the average rectal temperature rose to 40.5°C., an increase of about 3.5°C., while the average temperature in the mouth rose to 39°C., a rise of about 2°C. The rectal and mouth temperatures were also taken by means of mercury thermometers. All temperature readings were made while the current flow was discontinued.

The operation of the electrical circuit may be explained as follows: The radio-frequency potential of the electrode is raised to a value between the ground and that of the condenser plate adjacent to which a pick-up plate is suspended. Normally, the center of the body is at ground potential, hence, in this case, there must be a larger potential gradient from the electrode to the other condenser plate than would normally obtain in this region. Because of the small change in capacity of the system, a change in wave length was anticipated. Measurements of the wave length show that this change is of the order of one-half meter.

5920

Heterochronism in the Single Fiber Nerve-Muscle Complex of the Retrolingual Membrane.

HARRY GRUNDFEST. (Introduced by D. W. Bronk.)

From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania.

A series of measurements has been made on the excitability of the single muscle fiber preparation in the retrolingual membrane of the frog's tongue. The relation between liminal intensity and stimulus duration has been studied with the direct excitation of normal and curarized fibers and also with indirect stimulation through nerve fibers lying in the membrane. In many experiments it has been possible to carry out all 3 types of measurements while observing throughout the response of the same single muscle fiber.

The preparation is made according to the method described by Pratt,[1] which permits the use of transilluminated membranes with intact circulation. Stimulation has usually been by condenser discharges. The stimuli are applied through a localized cathode which is a capillary tube having an opening 20-80μ in diameter and which is filled with Ringer's solution.

These experiments were carried out to test the validity of Lapicque's[2] "law of isochronism" that transmission of excitation from a nerve to its muscle is possible only when the "chronaxies" of the 2 tissues are equal or differ by no more than a ratio of 1:2. That a block in transmission produced by drugs is due to an overstepping of this ratio. In particular, a curari block is supposedly due to an increase in the chronaxie of the muscle.

In the present experiments it has been found that the nerve fiber always has chronaxies from 2-8 times smaller than does the muscle fiber. The chronaxie of the muscle fiber does not change upon curarization. The values of the chronaxies range from about 0.03 to 0.06σ for the nerve and from 0.1 to 0.3σ for the muscle fiber. Furthermore, it has been possible to obtain compound curves of the general type described by Lucas[3] and Rushton[4] but, of course, with time values entirely different from theirs and corresponding with those given above. Since stimulation of the nerve produces a response in the entire motor unit, while on direct stimulation only the one muscle fiber responds, the 2 types of response appear entirely different to the observer. The discontinuity in the compound curve is therefore unambiguous. Because of this fact, it is also possible to obtain the suppressed branches of the 2 individual curves.

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[1] Pratt, F. H., *Am. J. Physiol.*, 1930, **93**, 9.

[2] Lapicque, L., *L'Excitabilité en fonction du temps.*, 1926.

These results are in conflict with the theory of isochronism expounded by Lapicque. On the other hand, they are in agreement with the view held by Lucas and by Rushton that the excitable substances of nerve and muscle are different and that this difference manifests itself in a condition of normal heterochronism. Such heterochronism can also be demonstrated in the membrane preparation which is not covered with Ringer's solution and which is stimulated with silver-silver chloride wire electrodes. Consequently, Lapicque's[5] latest objections to the view of Lucas and of Rushton disappear.

A more serious conflict seems to exist between my data and those of Moore and Brücke[6] which appeared after the above experiments were finished. They found chronaxies of 2.5-70σ for retrolingual muscle fibers and 0.9-1.5σ for the hypoglossal nerve trunk. I have repeated their experiments using a Ag-AgCl wire anode running the length of the tongue and perpendicular to the fibers. The cathode has been alternately a coarse Ag-AgCl wire point electrode and the usual capillary electrode. In one experiment both nerve and muscle fibers have been stimulated and compound curves obtained successively with the different cathodes. Using the coarse wire cathode, the chronaxie of the nerve fiber was 0.1σ and of the muscle fiber 2.1σ. With the capillary electrode, the chronaxies became 0.04σ and 0.12σ, respectively. The difference between my results and those of Moore and Brücke is, therefore, due to the effect of electrode size upon chronaxie which was found by Davis.[7]

No relation has been found between the chronaxie and the diameter of the muscle fiber in the region of the cathode. I cannot, therefore, agree with the attempt of Moore and Brücke to reconcile their data with Lapicque's theory. Both their experiments and mine seem to indicate that the theory of isochronism is not valid for the retrolingual preparation.

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[3] Lucas, K., *J. Physiol.*, 1907, **36**, 113.

[4] Rushton, W. A. H., *J. Physiol.*, 1930, **70**, 317.

[5] Lapicque, L., *J. Physiol.*, 1931, **71**, 189, 219.

[6] Moore, A. R., and Brücke, E. Th., *Pflüg. Arch. ges. Physiol.*, 1931, **228**, 619.

[7] Davis, H. W., *J. Physiol.*, 1923, **57**, Proc. lxxxi.

5921

Afferent Impulses from Single End Organs in the Carotid Sinus.*

D. W. BRONK and G. STELLA.

From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania.

Previous experiments revealed the general character of the discharge in the carotid sinus nerve and its relation to the pulse curve.[1] They showed a large burst of impulses resulting from the rapid rise in pressure at the beginning of systole followed by comparative inactivity during diastole. As the pressure increases the impulses become more continuous, extending throughout the heart cycle.

This work has been much extended and many observations have been made on the discharge of impulses from single end organs in the carotid sinus of the rabbit. Electrodes lead from the exposed nerve to a vacuum tube amplifier and thence to either a loud speaker or a Matthew's oscillograph for photographic registration. The procedure has been to divide the nerve in such a way that only one of the remaining fibers is in connection with a functionally active end organ. It is easy to decide when this stage in the dissection has been reached for either the sound furnished by the loud speaker or the photographic record from the oscillograph reveals a single series of regularly spaced impulses. The pulse curve has been recorded simultaneously by means of a Wiggers' manometer.

A typical record, Fig. 1, shows that with the beginning of the rapid rise in pressure the end organ starts to discharge impulses at a rate of about 55 a second, which then decreases as the pressure falls. The duration of this discharge in the heart cycle varies largely from one preparation to another and is obviously a function of the threshold of the end organ, the mean blood pressure and the form of the pulse curve. The first condition is well illustrated by preparations in which there are 2 or 3 active fibres remaining. In such cases one commonly finds one of the end organs continuing to function later in the cycle than the others.

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* The expenses of the research have been in part defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Bronk, D. W., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 1014.

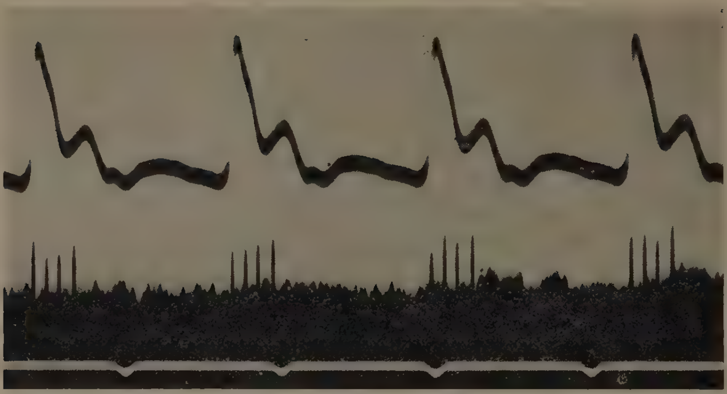

FIG. 1.

Nerve impulses from a single end organ in carotid sinus. Upper record: Blood pressure (mean 63 mm.); middle record: Oscillograph record of impulses; lower record: Time in 1/5 secs.

The effect of increased blood pressure is very definitely to increase the duration of the activity per heart cycle of a single end organ. From experiments in which the mean blood pressure ranged from about 40 mm. Hg. (amyl nitrite) to 150 mm. Hg. (adrenalin) we have obtained records of the corresponding nerve discharges showing no impulses at all, one per systole, then 2, 3, and more as the pressure increased until they were continuous with only slight variations in frequency corresponding to systole and diastole.

In preparations in which several fibres are active this increase in the level of blood pressure shows not only an increase in the frequency and duration of impulses from the individual end organ but also an increase in the number of end organs responding. Starting with a subthreshold pressure at which no endings are stimulated, first one and then another comes into action during systole as their several thresholds are reached. In the normal animal, therefore, as the blood pressure rises more impulses reach the centers in the medulla each heart cycle—as a result of an increased number of functionally active end organs, a longer duration of discharge from each end organ per cycle, and a higher frequency of discharge from the several endings.

Preliminary tests have been made to determine the effect of CO_2 on these receptors. Although the animals have rebreathed 10% CO_2 for over 3 minutes we have only observed a variation in frequency or duration of impulse discharge that could be accounted for merely by the accompanying change in level of blood pressure. This point, however, as well as the effect of other chemical agents is being more extensively investigated.

5922

Influence of the Hypersensitive State in Experimental Streptococcus Viridans Bacteremia.

DAVID WEISBERGER. (Introduced by Raymond Hussey.)

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While studying the localization of *Streptococcus viridans* at sites of local antigen-antibody reactions in rabbits sensitized to horse serum, it was observed that there was a difference in the rate of disappearance of these organisms from the blood stream when injected intravenously into normal animals and animals sensitized to horse serum. Bull[1] observed the fate of bacteria injected into normal dogs and rabbits. Bail[2] made similar observations, and Hopkins and Parker[3] and Wright[4] studied the fate of bacteria in normal and immunized animals. More recently Boone, Chase and Brink[5] made some similar observations working with intestinal absorption of *B. prodigiosus* in dogs during acute anaphylactic shock. There has not been found so far in the literature any work concerning the rate of disappearance of *Streptococcus viridans* from the bloodstream of hypersensitive rabbits.

A strain of *Streptococcus viridans* was isolated from an apical abscess of a tooth at necropsy. The hourly growth rate in broth was established. A determination of the quantity (pour plates) of organisms injected was made at the time of inoculation. The volume of the suspension in saline was kept constant at 1 cc. Inoculations were made into the marginal ear vein of the rabbits. Blood cultures, both pour plates and broth, were taken from the femoral vein at intervals of 15 minutes for the first hour, then half hourly for 3 hours, then hourly for 8 hours, and finally every 24 hours until the animal died or until the cultures remained negative.

Three groups of animals were used. Group A, normal animals, received injections of organisms only. Group B, normal animals, received horse serum and organisms, simultaneously. The sensitized animals (Group C) received both horse serum and organisms.

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[1] Bull, *J. Exp. Med.*, 1914, **20**, 237.

[2] Bail, *Arch. f. Hyg.*, 1905, **52**, 272.

[3] Hopkins and Parker, *J. Exp. Med.*, 1918, **27**, 1.

[4] Wright, *J. Exp. Path. and Bact.*, 1927, **30**, 185.

[5] Boone, Chase and Brink, Proc. Soc. Exp. Biol. and Med., 1931, **29**, 1.

Of 8 animals in Group A, 3 received 185 million organisms per cc. The remaining 5 received 10, 20, 100, 560 millions and 1 billion respectively. In Group B, 2 rabbits received 33 and 44 million respectively. In Group C, 4 animals received quantities 36, 44, 117, 200 million per cc. respectively. The amount of horse serum used was ½ cc. and 1 cc.

Results. Group A (Normal). In rabbits injected with quantities of organisms in the ranges of 10 million to and including 185 million, the blood cultures became negative after 1½ hours. When 560 million organisms were injected the blood cultures became negative only once at 2 hours and positive again until death. In rabbits receiving one billion organisms, the blood cultures remained positive throughout, septicemia resulting in death of animal within 74 hours.

Group B (Normal animals). The horse serum injected simultaneously with the organisms had no effect in prolonging the bacteremia. The blood cultures becoming negative at 1½ hours.

Group C (Sensitized animals). The blood cultures remained positive even though minimal doses of organisms were used. In some rabbits the cultures remained positive for as long as 72 hours.

The normal animals receiving doses in the range of from 10 million to 185 million gave cultures at the 15-minute period which had counts varying from 8 to 38 organisms per cc. of blood. In the sensitized animals, however, even those receiving inoculations of organisms within the same range, the 15-minute culture gave 400 organisms or over per cc. of blood. The only instance in which the 15-minute count reached a high number for normal animals was one which received an injection of one billion organisms; here the count rose to 439 organisms per cc. In sensitized and normal animals developing a prolonged bacteremia, there was a diminution of the count after the first 15 minutes. A low level was maintained from the second hour to the eighth hour when a secondary rise occurred progressing in some cases to 24 hours, after which the count diminished rapidly. In one instance only a septicemia developed. This was in the normal animal receiving an initial inoculation of one billion organisms.

5923

Method for Obtaining Curettings from Uterine Mucosa of Macacus Monkeys.

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A study of the uterine mucosa of the monkey has been facilitated by methods which permit examination of fresh samples of endometrium at intervals on the same animal with any given experimental history.

One method has been a surgical approach through the abdomen. The uterus is elevated and brought into the space made by the abdominal incision. A small soft rubber catheter is then passed around the lower uterine segment, just above the swelling of the cervix, and is tightened and clamped to form a tourniquet, effectively stopping the blood from the uterine arteries. This method is similar to that used by Dr. Squier in Hartman's laboratory, Carnegie Institute of Embryology, for operation on the pregnant monkey uterus.

After the circulation is stopped a small triangular wedge is taken from the fundus, the 2 incisions passing through the muscularis to the lumen of the uterus. The muscularis is closed by several stitches, the peritoneum drawn over and secured and the tourniquet released. The loss of blood is surprisingly slight and seepage may be stopped by sponging, or application of the piece of crushed muscle. The position of the removed section is indicated on a diagram and the operation may be repeated within a week, the wedge being taken from another portion of the fundus. This operation has been done several times on the same animal, and there has been no indication that the later sample had been adversely affected by the previous operations. Three portions have been taken from a uterus, but the method has obvious limitations.

Attention was then turned toward the possibility of obtaining scrapings adequate for study by a curettage. The first difficulty encountered was in the tortuous cervical canal in the monkey, which differs markedly from that in the human. The direct course is deflected by a projecting cervical colliculus. The cervix is relatively avascular as compared to the fundus. It has a large amount of collagenous fibers which make a relatively rigid structure in the nulliparous animal. In order to pass a curetting instrument into the fundus it is necessary to remove a portion or all of this cumulus.

A speculum was first used to distend the vagina, but this was discontinued as it was necessary to reduce the amount of unwieldy equipment in the field of work. Test tubes of graded sizes were cut off to the required length and slightly tapered on the cut end. One of these which would just fit in the pelvic outlet was inserted into the vagina, and forced into the vaginal fornices. This brings the cervical os nearly in the center of the field, and the pressure of the glass tube holds the cervix firmly, so that it is rarely necessary to grip it with forceps. For the removal of the cervical cumulus as well as for the subsequent curetting, a Spencer universal lamp was adapted. This lamp affords a spot focus, so that a bright beam of light may be focused directly on the cervical ostium. At the preparatory operation, with the animal on an inclined board in approximately a knee-chest position, a glass tube dilating the vagina and light in place, the cervical obstruction is quickly removed with a lancetlike blade. Bleeding has been stopped by sponge packing. This operation is unsatisfactory in that it must be done blindly, with only a sense of touch to direct the operation. No fatalities and, so far as is known, no perforations have resulted. A curettement may be made within a few days after this preparatory operation.

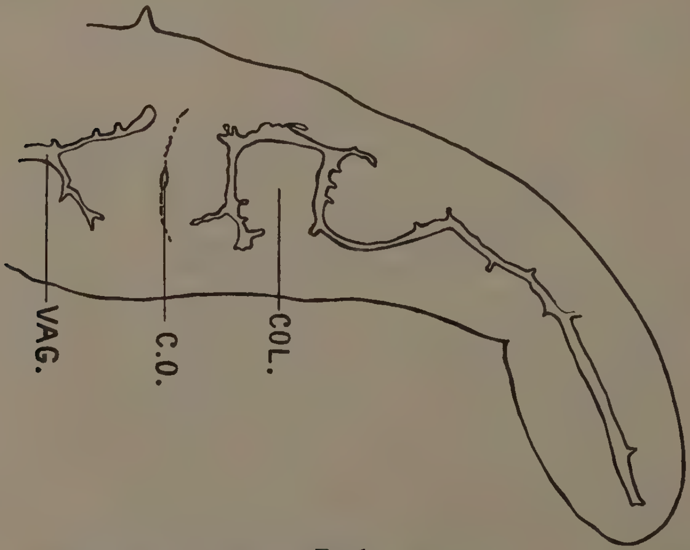
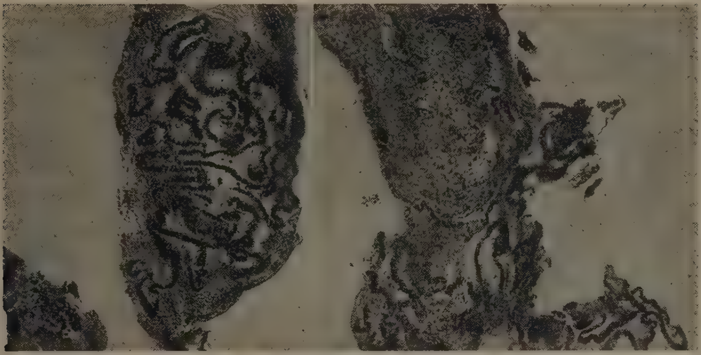


FIG. 1.
Projection tracing of uterus of immature *Macacus rhesus*. col., cervical colliculus; c.o., cervical os; vag., vagina.



FIGS. 2 AND 3.
Microphotographs of 2 samples of endometrium obtained by curettage.

In addition to the set-up described above several sharp curetting instruments are needed. The size of the instrument is limited only by the diameter of the os, as no method of dilating has been developed. Meyhoefer's eye curette has been found most satisfactory, although Buck's ear curette also has been used. The cervix must be cleaned of mucous, and the curette passed through the cervix. By placing the left hand on the abdomen of the monkey immediately cephalad of the pubis, the operator may feel the knife in the fundus and may exert slight external pressure at the time the tissue sample is being scraped from the endometrium. Unless the cervix has been cleared of mucous, the specimen will often be lost on withdrawal of the knife. Epithelium of the cervix is easily distinguishable from the endometrium.

The technique is not thoroughly satisfactory but does afford an opportunity for obtaining endometrial tissue (Figs. 2 and 3) without interfering with the continuance of an experiment.

5924

A Biological Method of Assay of the Adrenal Cortical Hormone.*

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Since extensive experimentation has failed to reveal a reaction of the normal animal to cortical hormone administration, it has become

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* Aided in part by a grant from the Josiah Macy, Jr., Foundation of New York.

increasingly clear that for the time being, adrenalectomized animals must be used for standardization purposes. There is no known criterion for determining accurately the degree of insufficiency from which an adrenalectomized animal is suffering, and consequently standardization cannot be based on the return of an animal suffering from adrenal insufficiency to a normal physiological condition. We have, therefore, accepted the other alternative, that is the maintenance of normal physiological condition in the adrenalectomized animal. During recent metabolic studies on bilaterally adrenalectomized dogs, permitted to go into adrenal insufficiency by the withdrawal of daily injections of cortical hormone, it was noted that the earliest indication from normality is a rise in the blood non-protein and in the urea nitrogen.[1] We have utilized this observation as a basis for biological assay of the extracts.

Adult dogs, 6 to 10 kg. body weight are selected, free of infection and worm infestation. They are adrenalectomized in 2 stages, and the success of the operation is established after complete healing of the wounds by production of insufficiency symptoms by withdrawal of extract. The dogs are then placed in separate cages and given 0.5 cc. extract per kilo in 2 equally divided doses daily, morning and evening, subcutaneously until it is demonstrated that the blood non-protein-nitrogen (or urea) is at a normal and constant level, that the weight is maintained, the food properly eaten, and the clinical condition in every respect normal. The ration given is constant and consists of 80 calories per kilo per day of Bal-Ra dog ration with 1 cc. cod liver oil 3 times weekly and 1 gm. vitavose per kilo 3 times weekly on alternate days. The dogs are permitted to run in open air weather permitting, at least 2 hours daily. When normal constant conditions are assured the per kilo dosage is progressively reduced at 7-10 day intervals, usually from 0.5 cc. per kilo to 0.25 cc., then to 0.13 cc. and so on until the blood non-protein-nitrogen shows a definite rise (15 mg. or to a level above 50 mg. per 100 cc.) followed by anorexia and the other clinical evidences of imminent adrenal insufficiency. Our stock extract (1 cc. represents 30 gm. beef adrenal cortex) prepared by methods previously described will maintain the animals in normal condition at a level of from 0.25 cc. to 0.1 cc. per kilo, the minimum dose varying with different batches of extract. At the present time we are using 2 animals for each assay. Several of our adrenalectomized dogs on assay work have been completely adrenalectomized for the past 6 to 10 months.

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[1] Harrop, Pfiffner, Weinstein and Swingle, *Science*, 1931, **73**, 683.

TABLE I.
Dog P-1. Second adrenal removed Jan. 17, 1931. Assay of Feb. 1 extract lot.

Date 1931	Weight kg.	NPN blood mg.%	Dose ext. cc. per kg. per day	Clinical condition.
Feb. 4	6.4	46	0.25	Lively. Eats well.
15	6.3	36	0.16	Lively. Eats poorly on 2/18 and 2/19.
20	5.9	60	0.12	Symptoms of severe insufficiency.
24	5.5	92		
25	5.3	44	2.0	Recovering. Food fairly well taken.
26	5.8	38	2.0	Lively. Eating well.

A dog unit is defined as the minimum daily kilogram dose of cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of 7 to 10 days; the two criteria of normal physiological condition being maintenance of body weight and blood level of non-protein-nitrogen (or urea). According to this definition the hormone content of different batches of our stock cortical extract varies from 4 to 10 dog units per cc.

Recently Kutz[2] has suggested the use of the adrenalectomized rat as a test animal since adrenalectomy is uniformly fatal in young rats of his colony. Obviously the use of a small animal form would have very definite advantages in standardization work, but the findings of various workers on the percentage mortality in the rat following adrenalectomy are so confusing that for the time being we prefer to use the dog. All workers are agreed that the occurrence of accessory tissue in the dog is exceedingly rare.

5925

Experiments on the Filtrability of the Rosenow Streptococcus of Anterior Poliomyelitis.

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This paper deals with the successful filtration through Berkefeld candles of 2 strains of the Rosenow anterior poliomyelitis streptococcus. These strains were designated 1630.30 and 1694^{2}.50. 0.5 cc. of a 20-hour brain infusion broth culture of 1630.30 was inoculated intracerebrally into each of 3 rabbits. Two of these animals died in less than 24 hours, and the third in 36 hours. Autopsies showed typical cerebral meningitis. From cultures of the brain, meninges, and cerebral exudate, the original streptococcus was recovered in each case. Heart blood cultures were negative.

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[2] Kutz, R. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 91.

The brains of these 3 animals were emulsified in broth and 0.5 cc. reinoculated intracerebrally into fresh rabbits. Death again resulted in approximately 18 hours, with recovery of the streptococcus. Animals inoculated with 0.5 cc. of the Berkefeld N filtrate of these emulsions suffered no ill effects. By intensive cultivation of these 3 brain emulsion filtrates using the Hauduroy transplant method on blood agar plates, we were able to recover from 2 of them an organism resembling both microscopically and culturally the 1630.30 streptococcus. In all cases it appeared on the 4th to 6th plate in the series.

Identically this same procedure was carried out using the 1694.50 strain Three rabbits were inoculated intracerebrally. All showed meningitis symptoms but only one died (48 hours). The streptococcus was recovered from brain cultures of this animal, but not from the heart blood. An organism believed to be 1694.50 was also recovered from the brain filtrate in the same way as for the 1630.30 strain.

Then each of the 4 strains was treated in the following manner: 20 cc. of a 24-hour brain heart infusion broth culture was injected intraperitoneally into a small half-grown rabbit and 50 cc. into a large rabbit. Eighteen hours later 50 cc. of broth was injected by the same route and after thorough manipulation of the abdomen the animals were killed and the peritoneal exudate removed aseptically and made up to a volume of 35 cc. with sterile broth. These lysates were then filtered through Berkefeld N candles and the filtrates cultivated on blood agar plates by the Hauduroy transplant method. On the 3rd to 6th plate, in every case, a streptococcus was recovered. This growth appeared at first as a very minute colony, not affecting blood, and closely resembling a pleomorphic diphtheroid upon microscopic examination. Further cultivation in each case gradually developed a streptococcus giving a faint greening action on blood and resembling the Rosenow strains in every way. Agglutination tests have not been carried out between these 2 strains. We do not consider it necessary that the streptococcus recovered from the filtrates be exhaustively tested for identity with the original Rosenow streptococcus. Very commonly filtrates do not yield immediately the identical organism and the crucial point here is not this, but whether the filtrable or virus form, so-called, is identical with the virus of polio.

At the same time that the animal inoculations were being performed a Berkefeld N filtration was made of a 24-hour broth (brain-heart infusion) culture of each of the strains as received from Rosenow. Prolonged plate to plate cultivation of these filtrates gave no growth. Each of the filtrates was then divided into 2 parts and sealed in ampoules. One part was incubated at room temperature and the other at 37°C. Five months later a slight sediment was noted in the filtrate of the 1630.30 strain which had been kept at 37°C. A slight opalescence was also noted in the $1694^{2}.50$ filtrate kept at the same temperature. Plates made from these ampoules at this time gave minute colonies—the 1630.30 filtrate grew on the first plate, while the $1694^{2}.50$ required from 2 to 3 serial transplants. These colonies likewise proved to be indifferent pleomorphic diphtheroids, but upon 3 days' growth in whole blood regained their streptococcus characteristics, and we believe them to be identical with the original strains filtered.

The filtrate samples kept at laboratory temperature have failed to give any growth with even the most careful and prolonged cultivation. As a result of these experiments we feel justified in concluding that these 2 strains of streptococci recovered by Rosenow from cases of anterior poliomyelitis in human beings are, at certain phases of their growth, filtrable through Berkefeld N candles.

Through the courtesy of Dr. C. W. Jungeblut of the Department of Bacteriology of the College of Physicians and Surgeons the filtrates from these 2 cultures were injected intracerebrally into monkeys with entirely negative results after a month's incubation. This would seem rather definite evidence that these filtrable forms are not identical with the virus of poliomyelitis. In fact, they probably have no cyclic relation to it, a view made conceivable by the discovery of filtrable forms of bacteria.

Nevertheless, the presence of these organisms in the disease, with which they react specifically in serologic and immunologic ways, still remains to be explained. One of us (Mellon) wishes to postulate a new point of view, which is a compromise between the 2 extreme views now held, viz, that the streptococcus is a pure secondary invader having no relation at all with the disease, and on the other hand the view that the streptococcus is but the visible stage of the causative virus.

My suggestion is that as a result of the altered conditions brought about in the host by the virus there is dissociated *in vivo* a form of streptococcus which reacts specifically with the host. I am led to make this suggestion partly as the result of a similar situation that has recently been shown to prevail in typhus fever and in small-pox. Fisher of this laboratory, in a study still unpublished, has shown that in hemorrhagic small-pox there is a hemolytic streptococcus in the blood with characters as specific for the disease as is the *Streptococcus scarlatinae* for scarlet fever.

Still more striking in this connection is the situation with Proteus X-19 in its relation to typhus fever. Silber[1] has shown that *B. proteus vulgaris,* which does not react serologically with the serum of typhus cases, can be made to do so as the result of its *in vivo dissociation* in collodion sacs in the abdominal cavities of typhus infected guinea pigs.

As knowledge of the biologic nature of the filtrable phases of bacteria increases it becomes clearer that their designation as viruses —for example, the tuberculosis virus—is apt to be misleading. On the other hand, the validity of these filtrable phases of bacteria is not in the slightest doubt, regardless of the specious explanations that have been raised regarding leaky filters, etc.

5926

Mass Cultures of Streptococcus Hemolyticus in Broth.

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One of us[1] found that a small amount of glucose appeared to be essential in media to be used for the cultivation of hemolytic streptococci and pneumococci. It seems likely that by a splitting of this substance to lactic acid, these organisms are able to derive energy for their growth. The amount of growth was roughly proportional to the percent of sugar, up to the point where sufficient acid was produced to check the growth of the organisms. This use of a phosphate buffered broth to delay the culture in reaching a toxic pH, thus allowing the utilization of more glucose and the development of heavier growth, is now very common. However, even a heavily buffered medium will yield not more than 2 or 3 times the volume of bacteria that can be obtained from the ordinary unbuffered infusion broth.

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[1] Silber, L., *Z. f. Hyg. and Infekt.*, 1928, **108**, 146.

[1] Mueller, J. H., *J. Bact.*, 1922, **8**, 309.

In the preparation of cultures of these organisms for securing large quantities of bacterial sediment or products, one might perhaps, by the occasional addition of sterile NaOH solution, keeping the medium constantly at a pH of 7.2 to 7.8, obtain considerably greater yields than from buffered broth. We have found this to be the case. Ordinary meat infusion broth containing 2% glucose and 5 or 6 drops of 0.2% phenol red solution to 100 cc. is inoculated heavily with a young, plain broth culture of a hemolytic streptococcus or a pneumococcus. After a few hours, when growth commences and the color begins to change from red to yellow, sterile N/1 or stronger NaOH is added from a capillary pipette until the proper red color returns, and incubation is continued. This process is repeated as often as necessary, perhaps every 15 minutes, as long as growth continues. With only 2% glucose this is usually 5 or 6 hours. With larger quantities of glucose it may be much longer, and go more slowly, perhaps because of osmotic effects or the accumulation of metabolic products. At the end of this time, streak plates prepared from such cultures show both streptococci and pneumococci to be still alive, whereas streaks from control unneutralized 0.5% glucose cultures which have become strongly acid are frequently dead. The yields of centrifuged streptococci are very much greater from the neutralized cultures than from controls. From 100 cc. of control culture one obtains ordinarily less than 0.1 cc. of packed sediment, which is usually a dirty gray color and undoubtedly consists in part at least of material precipitated from the broth by the acid formed. From 100 cc. of neutralized broth, approximately 0.5 cc. of white sediment is obtained, made up largely of live organisms.

In the case of pneumococci, autolysis apparently proceeds rather quickly, and the centrifuged bacteria are not so bulky. However, precipitin tests with type-specific sera show much higher concentrations of soluble specific substance in the neutralized cultures than in the controls.

It is a peculiar fact that streptococcus cultures grown in this way are often largely gram negative in smear, although perfectly viable.

5927

Immunization of Guinea Pigs Against Typhus Exanthematicus.

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Although many attempts have been made to discover a method of immunizing animals against *Typhus exanthematicus*, only a few isolated partially successful results have been reported.

Immunization has been accomplished with live virus mixed with immune serum[1] as well as with repeated injections of minute doses of live virus.[2] Immunization with heated tissues from infected animals has yielded negative results. Positive or partially positive results have, however, been reported with carbolized and formolized suspensions of Rickettsia. Breinl[3] has partially immunized guinea pigs with carbolized suspensions of infected lice stomachs containing large numbers of Rickettsia, while Zinsser and Castaneda[4] have recently reported successful results with formolized suspensions of Rickettsia from tunica material from guinea pigs infected with Mexican Typhus.

Our experiments were conducted with the European virus and we were concerned with the possibility of producing immunity with dead or attenuated virus in tissue suspensions. Suspensions of brain material from infected guinea pigs treated in a variety of ways with antiseptics, metallic sols and colloidal adsorbents yielded negative results. Positive immunization has, however, been obtained by 2 different procedures. These results, interesting in themselves, are significant also because of the theoretical problem involved.

We confirmed the work of Breinl[3] and succeeded in immunizing guinea pigs with formolized Rickettsia suspensions obtained from infected lice. The degree of immunity seems to depend on the quantity of suspension injected. No animals showed temperature above 39°C.

In other experiments brain suspensions were used. In connection with the colloidal adsorption experiments it was found that distilled water inactivated typhus virus after a relatively brief exposure. Virulent brain virus suspended in water becomes inactive in 6 to

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[1] Noguchi, H., *J. Exp. Med.*, 1923, **38**, 605.

[2] Nicolle, C., Sparrow, H., and Conseil, E., *Arch. Inst. Past. de Tunis*, 1927, **16**, 1.

[3] Breinl, F., *Z. für Immunitâts.*, 1924, **41**, 97.

[4] Zinsser, H., and Castaneda, Ruiz M., *J. Exp. Med.*, 1931, **53**, 325.

TABLE I.

G. P.	Material injected*	Date of inoculation	Date of infection	Material infected	Reaction	Lice infected with	Date
No. 913 W. 405 gm.	Emulsion 5 infected lice intestines in ¼ cc. saline + ¼ cc. 0.2% formalin, 24 hours in icebox. Intraperitoneal.	5/27/31	6/15/31	Brain material from infected guinea pig. 1 cc.	Typical	Brain G. P. blood	5/17/31 5/20/31
No. 944 W. 400 gm.	½ cc. suspension of 7 infected intestines + ½cc. of 0.2% formalin, kept over night in icebox. Intraperitoneal.	6/23/31	7/19/31	2 cc.	Incubation 8 days, duration of fever 4 days (modified course)	Infected lice intestines plus G. P. brain	6/15/31
	4 intestines in ½ cc. 0.1% formalin, kept over night in ice box. Intraperitoneal.	7/7/31				Lice rickettsia	6/30/31
No. 895 W. 440 gm.	5 injections: 17 intestines emulsified in 1 cc. 0.1% formalin, kept over night in icebox. Intraperitoneal, 0.3 cc. each.	7/20/31	8/5/31	½ cc. suspension	No fever; all 3 animals immune	Brain	7/10/31
No. 893 W. 400 gm.	4 intestines in 1 cc. 0.1% formalin. Intraperitoneal, 0.3 cc. each.	7/21/31				Brain	7/19/31
No. 967 W. 540 gm.	5 intestines in 1 cc. 0.1% formalin. Intraperitoneal, 0.3 cc. each.	7/22/31					
	5 intestines in 1 cc. 0.1% formalin. Intraperitoneal, 0.3 cc. each.	7/27/31					
	5 intestines in 1 cc. 0.1% formalin. Intraperitoneal, 0.3 cc. each.	7/30/31					
10 Control W. 400 gm. 11 Control W. 675 gm.			8/5/31	½ cc.	Typical course		

*The suspensions contained large numbers of Rickettsia.

8 hours and no longer produces an apparent infection in guinea pigs. Four to 5 successive injections of 0.5 to 1.0 cc. of a 4% brain suspension into guinea pigs immunized them against a reinfection with a dose of typhus virus which produced 100% infections in controls. A summary of these experiments is tabulated in Table II.

TABLE II.

No. of injections	No. of animals	Amount injected	Interval between infections	Duration of inactivation	Reaction	Result* of infection
		cc.	days	hours		
5	1	0.25-1.0	4-6	6	4 days' fever, max. 39.8°	Immune
5	1	0.5	4-6	3 times 6 2 " 5	No fever	"
4	1	0.5	5-6	2 " 6 2 " 4½	2 days' fever, max. 39.9°, after 3rd injection	" (intercurrent infection)
5	2	0.5	3-7	8, 7, 6, 5, 7	No fever	Immune
5	4†	0.5	4-7	7, 6, 5, 5, 4½	3 no fever; 1 small rise in temperature for 2 days; after 3rd injection	3 immune 1 typical infection

* In all cases controls infected at the same time with the same dose developed a typical infection after an incubation period of 6-7 days.

† 7 cc. mixed serum from these animals taken just before the infection protected a guinea pig against an infection with typhus virus.

Thus far carbolized or formolized brain material has failed to produce the same results, and it would appear, therefore, that in the case of the water suspension we are dealing with an attenuated virus. The interesting aspect of these 2 series of experiments is that the concentrated Rickettsia suspension behaves like a bacterial antigen, whereas the organ suspension reacts in the manner of a filterable virus; in the one case immunization can be effected by a presumably dead suspension of the infectious agent, and in the other it can be produced only by a live attenuated virus. This problem is now being studied more comprehensively, but the idea naturally suggests itself that the probable reason for the failure thus far to immunize with dead tissue virus, is the relatively small quantity of antigen present. If, as it appears, the degree of immunity produced is dependent upon the quantity of antigen injected, then there may be a simple explanation for the failure to immunize with dead virus suspensions.

5928

Action of Enzymes on Causative Agent of the Chicken Sarcoma.

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Our previous experiments[1] with the non-cellular filtrate of the Rous chicken sarcoma No. 1 suggest that the causative agent of the chicken sarcoma is a complex protein-like substance (globulin), or is associated with the globulin fraction. The present investigation was undertaken to secure further information concerning the true nature of the causative agent of the chicken sarcoma. As a first step, we determined whether the causative agent was inactivated by certain proteolytic enzymes.

Trypsin (Fairchild Bros. and Foster) and pepsin (U. S. P., 1-3000) were selected for the proteolytic enzymes and their protease actions tested upon the causative agent in tumor fragments and cell-free filtrates of the Rous chicken sarcoma at the hydrogen ion concentrations, pH 8.2 for trypsin and pH 3.8 for pepsin. The pepsin shows optimum action at pH 1.5. However, this optimum pH was not employed in the present investigation since our previous studies[1] with the Rous chicken sarcoma indicated that the causative agent of the R. C. S. did not survive in a fluid adjusted to this pH.

Five or more small pieces of tumor tissue, each weighing about 5 mg., were placed in 25 cc. portions of Locke-Ringer solution previously adjusted colorimetrically to the required pH values with NaOH and HCl, and kept at 4-5°C., and to which had been added 0.125 gm. of powdered trypsin or pepsin. The flasks containing the solutions and tumor fragments were allowed to remain for 24 hours in the refrigerator at a temperature of 4-5° C. The tumor fragments were then transplanted into chicks in the usual way. Each set of experiments included the inoculation of control animals with the tumor fragments which had been immersed in a Locke-Ringer solution at pH 8.2 or 3.8 for 24 hours at 4-5° C.

The results indicate that the transplantability of the R. C. S. is markedly affected by the action of trypsin and less markedly by pepsin. Of the 13 trypsin-treated grafts, only 2 developed slowly growing tumors, while of the 13 pepsin-treated grafts, 6 developed slowly growing tumors. In 16 control grafts, only one failed to develop a rapidly growing tumor.

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[1] Sugiura, K., and Benedict, S. R., *J. Cancer. Res.*, 1927, **11**, 164.

Gross examination of the tumor tissues immersed in a trypsin or pepsin solution for 24 hours at 4-5° C. showed the tissue to be slightly softened but unchanged in shape. The microscopic examination of these enzyme treated tumor tissues showed marked degenerative changes. Most of the tumor cells were distinctly smaller and looser. The intercellular substances were digested and giant tumor cells were absent. While most of the nuclei stained normally, there remained some cells which appeared microscopically to be normal viable tumor cells. The treated tissue is entirely devoid of giant cells, but still retains traces of viable fibrous elements.

The temperature which we selected for the present study was far from the optimum temperature for enzyme action. Therefore, as a control we determined the residual enzyme effect by injecting fresh tumor tissue together with a small amount of the enzyme solution. The results of this experiment showed that the growth capacity of the fragments of R. C. S. was not reduced by a single intratumoral injection of trypsin or pepsin solution.

When the fragments of tumor tissue are immersed in the trypsin or pepsin solution, a certain amount of the enzyme is probably absorbed in the tissue. Therefore, we attempted to remove the absorbed enzymes by washing the tumor fragments several times with fresh Locke-Ringer solution. The results showed that the number of takes and failure of the tumor grafts previously treated with trypsin and pepsin and subsequently subjected to washings are practically the same as that obtained from enzyme-treated unwashed tumor grafts.

The preceding study was extended to the cell-free filtrates of the Rous chicken sarcoma No. 1. Three aqueous extracts of fresh chicken sarcoma were prepared with 2.7, 5.2 and 10.5% of aqueous suspensions and freed from the tumor cells as before.[1] Portions of the filtrate were adjusted to the required pH values. To 25 cc. portions of the adjusted fluid 0.125 gm. of powdered trypsin or pepsin were added, shaken thoroughly, and kept 24 hours at 4-5° C. At the end of this period, from 1.0 to 2.0 cc. portions of the treated and untreated fluids were separately injected into 56 normal chicks, the treated fluid into the right and untreated control fluid into the left pectoral muscles. The results showed that none of the 28 chicks inoculated with the trypsin or pepsin-treated tumor fluid developed tumor. On the other hand, 26 of the 28 chicks inoculated with the untreated fluid developed rapidly growing sarcoma; the other 2 did not show tumor growth.

We next determined whether the heat-inactivated trypsin and pepsin will alter the capacity of the causative agent to excite tumor proliferation. In order to destroy the proteolytic energy, 10% solutions of trypsin and pepsin were subjected to a temperature of 80° C. for 15 minutes. To each 25 cc. of the adjusted original tumor fluid was added the inactivated trypsin or pepsin solution to an amount of 4.0 cc. Then the viability of the treated fluids was tested in the usual way. The injected chicks developed normally growing identical sarcomas.

During the course of the present investigation we also studied the effects of a castor bean lipase, a soy bean urease, and an amylase (Takadiastase) upon the causative agent in the Rous chicken sarcoma filtrate in a manner essentially similar to that used with the trypsin and pepsin experiments at the respective optimum pH values. The results showed that the lipolytic, deamidizing and amylolytic enzymes appeared to have no inhibiting effect on the causative agent since the majority of the injected chicks grew identical sarcomas.

Summary. 1. The causative agent of the Rous chicken sarcoma in the tumor fragments or in the cell-free tumor filtrate was inactivated by trypsin and pepsin, but was unaffected by the lipase, urease, or amylase preparations which were used as regards its capacity to produce tumor growth. 2. The causative agent was not destroyed if the proteolytic energy of trypsin and pepsin was abolished by heat inactivation. 3. Specificity of the proteolytic enzymes upon the causative agent of the chicken sarcoma suggests that the active agent is a protein or *associated in some way with protein material.*

5929

Avitaminosis. VIII. Effect of Vitamin B Deficiency on Concentration of Non-protein Nitrogen of Blood.*

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Since during advanced stages of vitamin B deficiency there is considerable tissue catabolism, it was thought of interest to investigate if in this avitaminosis there are any significant changes in the non-protein constituents of the blood of the albino rat.

The total non-protein nitrogen of the blood was studied in 8 pairs of rats on diets deficient in the vitamin B complex and in 2 pairs on rations deficient only in vitamin B. Weekly determinations were made. Litters were restricted to the same plane of nutrition as the pathological animals but in addition received either the vitamin B complex or vitamin B in the form of dehydrated Northwestern baker's yeast. In addition, 3 sets of animals were studied in groups of 4. The first one of each group received the vitamin B deficient diet (vitamin G having been supplied by autoclaved N. W. yeast); a litter mate received vitamin B but was restricted to the same amount of food and water as the pathological animal; a third litter mate received the same ration as the second, but water *ad libitum;* and the fourth litter mate received the same diet as the second, and food and water *ad libitum.* In these animals the concentration of the non-protein nitrogen, urea, and uric acid of the blood were determined once weekly.

The age of the animals was 50 to 68 days. The weight was from 65 to 91 gm. and the experimental period lasted from 14 to 155 days.

Summarizing the results on the vitamin B complex, there was an average concentration of 39.7 mg. non-protein nitrogen per cent in the pathological animals and 37.7 mg. per cent in the controls receiving vitamin B, but on the same plane of nutrition as the avitaminotic rats.

In the uncomplicated vitamin B groups there was a concentration of 46.7 mg. non-protein nitrogen per cent in the pathological animals as compared with 44.6 mg. per cent in the restricted controls. It is thus apparent that the rise of non-protein nitrogen in the blood of vitamin B deficiency in the albino rat was found to be too small to be considered of any significance, neither was there any appreciable rise in the concentration of urea nor uric acid.

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* Research paper No. 241, Journal Series, University of Arkansas.

It was rather surprising to find that losses to the extent of 30% in body weight did not produce any greater increase in concentration of non-protein nitrogen of the blood than 8 to 10%.

5930

Avitaminosis. IX. Influence of Vitamin A Deficiency on Albumin-globulin Ratio of the Blood of Albino Rat.*

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Since it is now generally recognized that infection accompanies vitamin A deficiency,[1] and since it is claimed that during infection there is a change in the albumin-globulin ratio of the blood,[2] due to the rise of the globulins, it was thought of interest to investigate if in this avitaminosis there is produced a shift in the albumin-globulin ratio of the blood of the albino rat.

A total of 18 animals were used in this study, 12 pathological and 6 controls. The micro methods used for the determinations of albumin and globulin were those perfected by Greenberg.[3] Weekly determinations were made for periods ranging from 63 to 102 days. The animals were 49 to 61 days old when started on the experiment and weighed 82 to 127 gm.

Summarizing all of our results for the entire experimental periods, the pathological animals showed an albumin-globulin ratio of 2.0 and the controls a ratio of 1.7. Only 4 animals out of the 12 pathological showed any considerable fall in the albumin-globulin ratio during the terminal stages of the avitaminosis, as indicated by a ratio of 1.3 in 2 animals and a ratio of 1.5 in 2 others.

From the character of our results we conclude that the albumin-globulin ratio cannot be used as an index of infection in vitamin A deficiency.

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* Research paper No. 242, Journal Series, University of Arkansas.

[1] Green, H. N., and Mellanby, E., *Brit. Med. J.*, 1928, **3537**, 691. Tyson, M. D., and Smith, A. H., *Am. J. Path.*, 1929, **5**, 57.

[2] Howe, P. E., *Physiol. Rev.*, 1925, **4**, 439.

[3] Greenberg, D. M., *J. Biol. Chem.*, 1929, **82**, 545.

5931

Sclerosing Substances. Effect on Vascular Endothelium.

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Injection of varicose veins has become the most popular form of treatment in this condition. Different sclerosing substances have been used to produce endothelial injury and thus cause an obliteration of the vein by the production of an intravascular coagulum.

We attempted to compare the relative effects of a group of sclerosing substances which have been employed clinically. That the results obtained are not exactly comparable with those obtained in the clinic is obvious, as the changes occurring in a normal vein following the injection of an irritating substance will undoubtedly be less marked than those occurring in an already diseased vessel. Twenty different sclerosing agents were injected into the internal saphenous or the cephalic veins of normal dogs. Sections were removed after one-half, one, 3, 6, 12, 24, and 48 hours; 3, 4, 6, 10, and 14 days; 3, 4, 6, and 8 weeks. Each section was examined histologically, employing hematoxylin and eosin; Weigert, Mallory connective tissue, and Sudan III stains.

The amount of endothelial injury varied considerably from complete destruction to little or no change. Almost invariably, the greatest amount of destruction was noted within the first hour. Whereas most of the sections removed at one-half hour to 4 day intervals showed rather extensive change, those sections removed after 4 days showed relatively little change, indicating a marked tendency toward regeneration between the fourth and sixth days. In spite of extensive endothelial injury as evidenced within the first 4 days, the incidence of thrombus formation was relatively low as only 28 fibrinous coagula and 11 fibrous, or organized, coagula were found.

The average amount of destruction produced by the different sclerosing substances in order of their severity is shown in Chart I.

All except sodium salicylate, 15%, invertose, 50%, glucose, 66%, and phenol, 1%, produced fibrinous coagula. Glucose, 66%, invertose, 50%, mercuric iodide, 1%, phenol, 1%, invertose, 50%, and sodium salicylate, 10%, and invertose, 75%, and sodium salicylate, 20% developed fibrous coagula. The greatest number (3) of organized or fibrous coagula were caused by sodium salicylate, 40%. Sodium salicylate, 30%, and sodium chloride, 10%; quinine

CHART I.
Average Destruction of Endothelium.*

Solution Used.	Average Destruction %
Sodium salicylate, 40%	58.7 ± 2.33
Quinine and Urethane	58.1 ± 2.64
Sodium salicylate, 30%, sodium chloride, 10%	53 ± 2.28
Sodium chloride, 25%	48.4 ± 1.89
Sodium salicylate, 30%	41.8 ± 2.21
Bichloride of mercury, 1%	33.6 ± 2.34
Sodium chloride, 20%	33.4 ± 1.24
Invertose, 75%	31 ± 2.51
Calorose	28.6 ± 1.78
Invertose, 75%, sodium salicylate, 20%	28.5 ± 1.29
Glucose, 66%	27.6 ± 1.54
Invertose, 50%, sodium salicylate, 10%	27.1 ± 1.12
Biniodide of mercury, 1%	19.1 ± 0.96
Iodine, 1%, potassium iodide, 1%	13.1 ± 1.52
Phenol, 1%	12.1 ± 1.33
Sodium salicylate, 20%	9.8 ± 2.2
Sodium salicylate, 15%	8.7 ± 1.99
Glucose, 50%	8.1 ± 0.65
Invertose, 50%	6.2 ± 1.85
Sodium chloride, 15%	1.8

*The average in each instance is based on 16 observations each made one-half, one, 3, 6, 12, 24, and 48 hours; 3, 4, 6, 10, and 14 days; 4, 6, and 8 weeks after intravenous injection of sclerosing substances.

and urethane; mercuric chloride, 1%, each produced 2 fibrous coagula; whereas invertose, 75%, and calorose each was responsible for the production of an organized thrombus. Canalization occurred in all the fibrous coagula.

Conclusions. Whereas the destruction of endothelium is responsible for the development of sterile thrombi following injection of sclerosing substances, the extent of the destruction does not necessarily indicate the degree of thrombosis. In spite of extensive destruction of endothelium, coagulation occurs relatively infrequently, but usually between the fourth and sixth days a regeneration of the endothelium occurs so that the vessel again becomes lined with a normal endothelium. This and the fact that canalization invariably occurs in the artificially produced thrombi possibly account for recurrences following the injection treatment of varicose veins.

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Production of Osteitis Fibrosa with Overdoses of Vitamin D.

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Irradiated ergosterol in corn oil was used as a source of vitamin D in the production of *Osteitis fibrosa* in guinea pigs. Seven groups of adult guinea pigs were given graded doses of activated ergosterol daily. Each group contained 7 pigs weighing in the neighborhood of 500 gm. One group was kept as a control. Complete studies of the serum calcium, inorganic phosphorus, and protein were made previous to autopsy. Sections of the long bones and the chondrosternal junctions of the ribs were fixed in Zenker's fluid and stained with phloxine and methylene blue.

No changes were present where small doses (0.75 mg. to 2 mg. daily) were used. The earliest change visible was a thickening of the fibrous layer of the periosteum of the long bones in the group that had received 5 mg. of irradiated ergosterol.

Changes simulating an *Osteitis fibrosa* were first seen in the group that received daily doses of 10 mg. of irradiated ergosterol. In 14 days slight fibrous stimulation was present with ingrowth into the marrow cavity. At 25 to 28 days there was resorption of the bony trabeculae together with an increase in the size of the lacunar spaces. These were lined by numerous osteoclasts and showed the presence of fibrous connective tissue ingrowths. The chondrosternal junctions of the ribs were thickened and the osseous portions showed fibrous changes similar to those seen in the long bones.

The group that received 15 mg. daily showed the greatest degree of fibrous change and presented a picture similar to that reported by Jaffe and his workers following the administration of parathormone. In 5 days the chondrosternal junction of the ribs showed fibrous proliferation. With the advance of time in the administration of the oil the bones showed thinning of the corticalis, widespread fibrous connective tissue replacement, and areas of hemorrhage in the marrow cavities. In 36 days resorption of the hemorrhagic areas were seen with the production of cyst-like spaces.

The symptoms manifested by the animals were those of *Osteitis fibrosa cystica* (von Recklinghausen) rather than of a toxic hypercalcemia. Loss in weight, apathy, and muscle weakness were marked. No gastro-intestinal disturbances were present. The highest rise in the blood calcium occurred in one of the 15 mg. pigs, which reached 13 mg. per 100 cc. of blood. The production of *Osteitis fibrosa* in the guinea pig by the use of toxic doses of activated ergosterol was probably due to the ability of that animal to eliminate calcium with sufficient rapidity to prevent a fatal hypercalcemia. The animals were carried along until a true *Osteitis fibrosa cystica* was produced. Additional groups of animals on basal diets, receiving toxic doses of ergosterol are now under study.

The similarity of vitamin D and of the hormone of the parathyroid gland in the production of *Osteitis fibrosa* appears to be further strengthened by these observations.

5933

Magnesium and Potassium Anesthesia in Amoeba.*

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It is well known that the magnesium ion may serve as an anesthetic for many organisms. Magnesium salts are generally considered the best of all anesthetics for most types of marine invertebrates, and are also used to anesthetize the tissues of higher animals. Potassium likewise serves as an anesthetic, especially for certain types of muscular tissue. It is therefore evident that 2 of the commonest cations of the living substance may prevent its activity. Concerning the nature of the action of magnesium or of potassium, there is almost no information, and the usual theories of anesthesia offer little help.

Both magnesium and potassium ions act as anesthetics for the common *Amoeba proteus.* Of the 2, potassium has the more pronounced anesthetic action. When amoebae are placed in dilute solutions of potassium salts, the pseudopodia are retracted, the amoebae round up, and movement ceases completely. In solutions of magnesium salts, the effect is not so pronounced. When amoebae are placed in dilute solutions of magnesium chloride or magnesium sulphate (after a preliminary washing in the solution), movement apparently ceases. Actually a very slow change in form may persist, but there is nothing that even approaches in speed the normal

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* Aided by a grant from the Research Committee of the University of Pennsylvania.

amoeboid movement, and one must watch for minutes at a time to see any movement at all. (It should perhaps be noted that *Amoeba dubia* is much more resistant to the magnesium ion than *Amoeba proteus.*) When amoebae are removed from potassium and magnesium solutions and placed in balanced solutions, they recover their power of movement. Both potassium and magnesium are antagonized by calcium.

It can be clearly demonstrated that both potassium and magnesium ions have a very real effect on the physical properties of the protoplasm. Amoeba protoplasm doubtless consists of an inner fluid plasmasol and an outer more rigid plasmagel. The physical properties of both these regions may be studied with the aid of the centrifuge method of determining protoplasmic viscosity, by making observations on the 2 common species of free-living amoebae, *Amoeba proteus* and *Amoeba dubia.* These two species look very much alike, but *Amoeba dubia* has larger crystals which are not held in the plasmagel but move readily when the amoeba is centrifuged at slow speeds. The action of the common cations on the protoplasm of *Amoeba dubia* has already been reported.[1] Calcium and magnesium tend to make it more fluid, potassium and sodium have the opposite effect. These results must be taken as applying to the plasmasol, and they show no correlation with the anesthetic action of the ions, for the 2 anesthetic ions have opposite effects on the protoplasmic viscosity of the plasmasol, and calcium and magnesium act in the same fashion.

The plasmagel can conveniently be studied in *Amoeba proteus.* When this amoeba is centrifuged, the large granules or crystals of its protoplasm move very readily through its interior, even when only relatively weak centrifugal force (*i. e.,* 128 times gravity) is applied. But the crystals in the cortical layer of the protoplasm do not move readily unless much more vigorous centrifugal force is applied. When the amoeba is subjected to a force approximately 4000 times gravity, the crystals of the cortex are displaced into half of the cell in about 80 to 100 seconds, the actual number of seconds varying with the culture and with external conditions. For an amoeba anesthetized in M/40 potassium chloride solution, this time becomes very much reduced. A large number of tests has shown that for such an anesthetized amoeba the time required for the shifting of crystals is only, on the average, one-eighth of that required for the normal controls. It seems certain that the potassium ion has produced a liquefaction of the outer cortex or plasmagel of the amoeba.

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[1] **Heilbrunn, L. V., and Daugherty, K., *Physiol. Zool.*, 1931, 4, 635.**

The magesium ion has a similar effect, but it is not nearly as pronounced. An average of 12 tests on amoebae immersed in dilute solutions of magnesium chloride showed a 32% decrease in the "centrifuge value", this value being defined as the time required at a given centrifugal force to produce the required shifting of crystals. Dilute solutions of calcium salts increase the centrifugal value.

It may be concluded that the anesthetic action of potassium and magnesium ions on the amoeba is associated with a liquefaction of a specific region of the protoplasm, a region which is presumably responsible for the amoeboid movement. We thus have clear support for the view that anesthetic action is associated with liquefaction, a view which I first stated in 1920, and for which additional evidence was cited in a recent monograph.[2]

5934

External Evidence of Hormone Action Following Injection of Urine of Pregnant Women into Rabbits and Guinea Pigs.

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These experiments were undertaken in an effort to find a manifestation of the presence of the pregnancy hormones that would not involve operating on an animal.

The speed and accuracy of the Friedman test pointed to the rabbit as the animal of choice. Vaginal smears were made on 6 female rabbits for a period of about a month. However, it was noted that there was no regularity in the appearance of the various types of cells, and that the injection of the urine of a pregnant woman, while producing the characteristic ovarian changes, did not affect the vaginal smears. This confirms the work of Kunde and Proud.[1]

Part of the explanation may lie in the anatomy of the rabbit. A dissection revealed that the urethra opens into the vagina at some distance from the vulva. Bladder epithelial cells may be washed into the vagina with the urine; a platinum loop will pass as easily into the bladder as further into the vagina. Smears made from vaginal washings showed very little difference in composition from those made with a loop.

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[2] Heilbrunn, L. V., "The Colloid Chemistry of Protoplasm." Berlin, 1928.

[1] Kunde, M. M., and Proud, T., *Am. J. Phys.*, 1929, **88**, 446.

The guinea pig was then investigated. This animal possesses a membrane which seals off the vagina except during oestrus and parturition.[2] A series of immature guinea pigs was injected with the urine of a pregnant woman near term. One received 1 cc. intraperitoneally twice a day, the other 2 received subcutaneous injections of the same amount once a day and 3 times a day respectively. In all 3 of these animals it was noted on the third day of the experiment that the vaginal membrane had ruptured spontaneously. The smears of the 2 animals receiving the larger doses showed typical proestrus cells. The only guinea pig which survived the next morning showed metoestrus cells in the vaginal smear. Autopsy on all 3 showed no ovulation but there was definite hypertrophy of the uterus, most marked in the animal receiving the massive subcutaneous injections. Male urine and the urine of a woman just starting to menstruate gave negative results.

Oestrin is probably responsible for this phenomenon. To see what part prolan might play, animals were injected subcutaneously with a suspension precipitated from the urine of a pregnant woman with alcohol and washed free of oestrin with ether. The amounts injected were equivalent to 40 cc. of urine. No changes were noted. Amniotin (Squibb) was injected into an immature animal, an amount corresponding to 240 mouse units, in divided doses, also with negative results. When the experiment was repeated, on 2 different series of animals, the animals died on the second and third day. In the original experiment 2 of the guinea pigs died on the fourth day. Evidently the urine is highly toxic to guinea pigs, especially young ones. In critically reviewing the data it is felt that most of the animals used were far too young, inasmuch as the various phenomena of the pregnancy hormones are most striking in animals close to maturity, and efforts are now being made to obtain guinea pigs of good stock whose age is known exactly.

This work, therefore, calls attention to the unreliability of the vaginal smears of rabbits, and to the opening of the vaginal membrane of immature guinea pigs when injected with the urine of a pregnant woman as indicating the presence of the pregnancy hormones.

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[2] Stockard, C. P., and Papanicolaou, G. N., *Am. J. Anat.*, 1917, **22**, 225.

5935

Lens Induction in Ectoderm Transplanted from Rana Palustris to Amblystoma Punctatum.

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Geinitz[1] showed that, if the dorsal lip of the blastopore is transplanted from an anuran gastrula to the gastrocoel of a urodele, it not only differentiates into typical axial organs but also induces the formation of a neural tube and somites in the urodele tissue with which it is in contact. Lewis[2] attempted to obtain lens induction by implanting an anuran optic vesicle beneath epidermis in the region between the eye and the ear of an Amblystoma embryo but he himself was not sure of the source of the lenses which developed after this operation. Since Geinitz's demonstration of xenoplastic induction, renewed attempts have been made to show that lens formation can be induced by xenoplastically transplanted optic vesicles. Cotronei and Spirito[3] and Mangold[4] have performed experiments bearing on this subject but have not obtained positive results in the species which they used.

In the experiments here reported, *Rana palustris* and *Amblystoma punctatum* were used as donor and host respectively. The operation involved removing epidermis from over the left optic vesicle of the urodele host at stage 22-23 and substituting for it a piece of head epidermis from the anuran donor. The donor, which was at approximately the same stage of development as the host at the time of operation, was, however, considerably smaller than the host. Therefore, in order to cover the host's optic vesicle, it was necessary to transplant an extensive piece of the donor's head epidermis.

Animals which had been operated upon were preserved at various stages during embryonal and early larval life, *i. e.*, from 4 to 23 days after operation, and were subsequently examined histologically. Xenoplastic lens induction was found to have taken place in 11 cases. Since the nuclei of anuran cells are markedly smaller than those of urodele cells, the discrepancy between the sizes of the nuclei in the Rana and Amblystoma lens epithelium furnished a reliable criterion for determining from what source a lens was derived even after the stage at which it had separated from the epidermis.

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[1] Geinitz, B., *Roux' Arch. f. Entwicklungsmechanik*, 1925, **106**, 406.

[2] Lewis, W. H., *Am. J. Anat.*, 1907, **7**, 270.

[3] Cotronei, G., and Spirito, A., *Atti della Reale Accademia Nazionale dei Lincei*, 1930, **12**, 70.

[4] Mangold, O., *Ergebnisse der Biol.*, 1931, **7**, 277.

The results of these experiments fall into 2 groups. The first includes those cases where only one lens developed in association with the Amblystoma eye over which Rana epidermis had been placed. These lenses were quite normal in shape and structure. They showed none of the signs of histolysis which Lewis[5] and Cotronei and Spirito[6] have found in xenoplastically transplanted optic cups.

The second group includes cases where 2 lenses, one of Rana and the other of Amblystoma origin, developed in association with the Amblystoma eye over which Rana epidermis had been placed. In the light of Cotronei and Spirito's demonstration that xenoplastically transplanted organs, histologically similar, have a definite affinity for one another and tend to fuse, particular interest attaches to one of the animals in this group. In this animal actual fusion took place between the Amblystoma and Rana lenses which were developing in association with the same Amblystoma eye. The lens fibers of the 2 components of the composite lens which was thus formed were contiguous and the uninterrupted sheet of epithelium surrounding the lens showed, by the sizes of its nuclei, that it consisted partly of urodele and partly of anuran cells.

The results of these experiments show that the eye rudiment of a urodele embryo can induce lens formation in anuran epidermis with which it is in contact and that the lens which is thus induced maintains its own histological characteristics.

A few operations were done in which *Amblystoma punctatum* epidermis was transplanted to the eye region of *Rana palustris*. In these cases no lenses developed since the grafts became necrotic very soon after the operation.

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[5] Lewis, W. H., *Am. J. Anat.*, 1907, **7**, 270.

[6] Cotronei, G., and Spirito, A., *Atti della Reale Accademia Nazionale dei Lincei*, 1930, **12**, 72.

5936

Thermal Changes in Denervated and Sympathectomized Limbs With and Without Arterial Ligation.

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The skin temperature obtained by inserting the bulb of a thermometer between the toes of dogs was found to vary with the room temperature. Since the response to changes of room temperature was greatly delayed in long haired animals, those having short hair were used. It was found necessary to keep the animal 15-30 minutes in a new environmental temperature before the skin temperature became stabilized.

Mosser and Taylor[1] reported that alcoholic injection or crushing of the sciatic nerve in dogs resulted in a constant hyperthermia. In repeating their experiments that year we found that this hyperthermia was only relative, being entirely dependent on the room temperature. Apparently from their protocols the skin temperatures were taken in a cold room.

When normal short haired dogs were kept in a warm room (23-30°C.) the temperature between the toes was from 36-39°C. In a cool room (17-22°C.) the skin temperature fell 1-6°C., falling lower the cooler the room. In a cold room (13-17°C.) the skin temperature was 6-14°C. lower than that obtained in a warm room. In a freezing room (3-6°C.) the skin temperature fell to 8-18°C. and occasionally the rectal temperature fell about one degree.

These results showed that the dog had a thermal vasodilation level of 36-39°C. when kept in a warm room, and a vasoconstriction level of 22-26°C. when kept in a cold room. When the room temperature approached freezing the further decrease of skin temperature was apparently due to a direct effect on the blood vessels.

Section or Crushing of the Sciatic Nerve. (14 experiments.) The interruption of the peripheral nerves to an extremity by cutting or crushing the sciatic nerve produced very little difference in the temperature (0-2°C. higher on the operated side) when the dog was kept in a warm room. In a cool room the operated side showed a relative hyperthermia of 3-6°C., the rectal temperature remaining constant. When the animal was placed in a cold room a marked relative hyperthermia (4-10.5°C.) existed on the denervated side.

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[1] Mosser, W. B., and Taylor, K. P. A., *Arch. Surg.*, 1926, **12**, 760.

That is, when the dog was taken from a warm room to a cool room, the skin temperature of the denervated limb remained about the same, but the temperature of the normal limb fell 3-6°C. In a cold room the temperature of the normal limb fell 4-10°C. When the dog was kept in a freezing room of 5°C. for one-half to one hour, the rectal temperature remained unchanged but both the limb temperatures fell to a level of 10-16°C., the thermal change being slower on the operated side. On returning to the warm room recovery to the original temperature was more rapid on the denervated side.

The denervated limb maintained its skin temperature at or near the vasodilation level in all environmental temperatures to a point as low as 13°C. As this effect was apparently due to the interruption of the vasomotor fibers in the sciatic nerve (Potts[2]) the lower lumbar sympathetic ganglia on one side were removed in other animals.

Unilateral Lumbar Sympathetic Ganglionectomy. (9 experiments.) Thermal changes similar to those obtained following section of the sciatic nerve were obtained when the second, third and fourth lumbar ganglia were removed on one side (method of Adson[3]). In a warm room the skin temperatures of both limbs were the same or the sympathectomized side showed a relative hyperthermia of 0.5-1°C. In a cold room no change or a slight fall (0-2°C.) occurred on the operated side, while on the normal side a fall of 5-13°C. was recorded. In the freezing room (5°C.) for one hour the temperature of both limbs fell to 10-16°C. (Fig. 1.) When

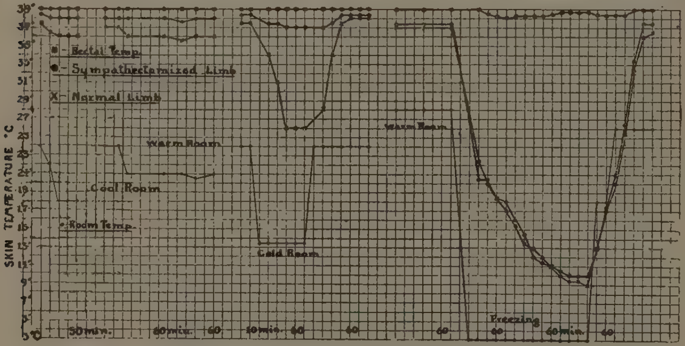

FIG. 1.
Effect of environmental temperatures on the skin temperatures of normal and sympathectomized limbs.

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[2] Potts, L. W., *Anat. Anz.*, 1914, 138.

[3] Brown, G. E., and Adson, A. W., *Am. J. Med. Sc.*, 1925, **170**, 232.

the hind feet up to the ankles were placed in ice water (8°C.) for 3 minutes (Lewis[4]) with the animal in a warm room, the temperature of the hind limbs fell to the levels obtained in the freezing room. Within 10 minutes after immersion the temperature on the sympathectomized side had returned to the original level while on the normal side it was 9°C. lower than its original level.

Periarterial Sympathectomy. (3 experiments.) When periarterial sympathectomy was performed on the femoral artery of a dog practically no thermal difference was noted. In a warm room the skin temperature was the same in both limbs. In a cold room the limb temperatures fell symmetrically about 10°C., the rectal temperature remaining the same.

Division of Femoral Artery Distal to Profunda Branch. (2 experiments.) When the femoral artery was ligated and divided distal to the profunda branch, observations during the first 4 post-operative days showed no thermal difference when kept in a warm room. Placed in a cold room the temperature of both limbs fell, that on the ligated side being slightly lower (0.5-1°C.). In the freezing room the fall in temperature was uniform.

Division of Femoral Artery Proximal to the Profunda Branch. (4 experiments.) In a warm room the temperature of the ligated side was 2-5°C. lower than the control, while in a cold room the ligated limb was 5-9°C. lower. Placed in the freezing room the temperature of the operated limb quickly fell 4-7°C. lower than the normal side and the recovery was much slower when returned to a warm room. After the seventh post-operative day only a slight thermal variation (0-2°C.) existed between the limbs.

Division of Femoral Artery Proximal to the Profunda Branch and Lumbar Sympathetic Ganglionectomy. (2 experiments.) Ligation of the artery and a lumbar sympathectomy on the same side produced thermal readings 2-4°C. lower than the normal side when the dog was in a warm room. In a cold room the temperature of both limbs fell, that on the operated side being 1.5-2.5°C. lower. In the freezing room both temperatures fell symmetrically with the operated side 2-4°C. lower. Recovery was at the same rate in both limbs.

Division of Femoral Artery Distal to Profunda Branch and Section of the Sciatic Nerve. (3 experiments.) After section of the main artery and nerve to a limb a relative hyperthermia (2-6°C.) existed on the operated side when the dog was kept in a warm room. In a cold room the thermal readings in both limbs fell, that on the operated side being 3-6°C. lower. In the freezing room both temperatures fell, but slower and to a lesser degree on the operated side. Recovery was slightly more rapid in the operated limb. After the eighth post-operative day the thermal responses were similar to those following section of the sciatic nerve alone.

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[4] **Lewis, T., *Heart*, 1929, 15, 8.**

Division of Both Femoral Arteries Proximal to the Profunda and Unilateral Lumbar Sympathetic Ganglionectomy. (2 experiments.) When the femoral artery to each hind limb was ligated and a unilateral lumbar sympathectomy performed the temperature of the toes on the sympathectomized side was 2-3°C. higher in a warm room. During the first half hour in a cold room the temperature fell in both limbs, that of the sympathectomized side being 2-8°C. higher. During the second half hour both limbs reached a new lower thermal level with the sympathectomized side 2°C. higher. When both hind feet were placed in ice water (8°C.) for 3 minutes, there was no thermal change in the rectum or fore limbs. The hind limbs showed a marked hyperthermia compared with the thermal readings of the foreleg; on the sympathectomized side the fall was 19.5°C. and on the other side 17°C. Recovery was slow and parallel in both limbs, about one hour elapsed before the temperatures returned to the original level.

Summary. Skin temperatures taken between the toes of dogs varied with the temperature of the room. The thermal vasodilation level of the normal limb of a short haired dog in a warm room was 36-39°C. The thermal vasoconstriction level was apparently 22-26°C. Section of the sciatic nerve or lumbar sympathectomy caused a relative hyperthermia in the limb of the operated side in dogs kept in a cold room. Following division of the femoral artery thermal responses paralleled those of the normal limb but were consistently slightly lower. Division of the femoral artery and ipsolateral lumbar sympathectomy or section of the sciatic nerve led to thermal responses to heat and cold similar to those obtained after division of the femoral artery alone. After division of the femoral arteries and unilateral lumbar sympathectomy the thermal responses were similar to, but more moderate than those after sympathectomy alone.

5937

Etiology of Poliomyelitis: Cultivation of an Organism from Poliomyelitic Tissue and Transmission of the Disease in Monkeys.*

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Bacteriological cultivation experiments were attempted with Berkefeld N filtrates prepared from the nervous tissues of monkeys inoculated with poliomyelitis virus and developing typical experimental poliomyelitis. Seven strains of virus were studied in a special culture medium designated as VB medium and containing essentially minced sheep brain in a veal infusion broth free from peptone. The use of fresh living tissue and ascitic fluid such as characterize the Noguchi medium or its modifications was avoided. No attempt was made to obtain strict anerobiosis by means of the recognized methods. The reduced oxygen tension developed in the culture medium was found satisfactory. The use of brain tissue suggested itself as a suitable culture medium for a virus that is known to exhibit a marked affinity for the nervous system.

The culture medium was prepared as follows: Blood and membranes were carefully removed at autopsy from fresh sheep brain. One kilogram of tissue was added to one liter of veal infusion broth, pH 8.0, free from peptone. The mixture was coagulated thoroughly by boiling for 2 to 3 minutes and 0.2% glucose by volume was added. The cooled material was then passed 2 or 3 times through a meat chopper. The culture medium was placed in test tubes measuring 1.5 by 15 cm. to a depth of approximately 6 cm. (about 10 cc. by volume) and sterilized in the autoclave at 15 pounds pressure for 20 minutes. The final pH was adjusted (if necessary) to 7.6 Rigorous tests for sterility of the material were made before use to rule out all possible sources of contamination.

The inoculum, consisting of Berkefeld filtrates as already described, was added to a series of tubes of culture medium and incubated at 37.5 to 38.0°C. for a period of 3 to 4 weeks during which time samples were withdrawn every week or 10 days by means of a platinum loop or capillary pipette for subplants and film preparations. Two tubes in each series were left unmolested and checked after growth had occurred in the series under observation. Control

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series included Berkefeld filtrates and suspensions of poliomyelitic tissues inoculated into ordinary fluid and solid culture media; filtrates and suspensions from normal monkey brain and cord; suspensions and filtrates prepared from nervous tissues in herpes encephalitis, and neurovaccinia; and finally uninoculated VB medium by itself.

Positive cultures were obtained from poliomyelitis virus filtrates and the transfers from the original cultures have yielded successful growth in series. At the present time these organisms are in the fourteenth subplant and the original cultures are still viable after growing in the incubator for more than 18 weeks.

The organism grows in an atmosphere of reduced oxygen tension developed in the medium, stains best with Wright stain and in later subplants and older cultures it appears to be faintly or moderately Gram-positive. Under a magnification of 1500 to 2000 diameters it is a minute ovoid body ranging in size from the lowest limit of visibility to 0.05-0.1 or 0.2 M, and occurring in irregular clusters, in pairs, singly, very rarely in short chains, and in densely packed masses. The bodies are especially numerous in the particles of brain tissue found in the medium, for which they exhibit a marked preference. A sheath-like envelope appears to surround the organism, favoring the formation of zoogleal masses. Filtration experiments with the cultures during certain stages of growth suggest that ultramicroscopic forms are capable of developing into visible bodies in the culture medium.

Inoculation in monkeys of organisms obtained from cultures of poliomyelitic tissues in VB medium caused clinical symptoms and pathological effects characteristic of experimental poliomyelitis. Successful experiments have been accomplished with these cultures representing to date a dilution of about 2×10^{-27} of the original material cultivated. This was 0.1 cc. of a 5% saline Berkefeld N filtrate of poliomyelitic nervous tissue. The cultures used were in the 6th to the 13th subplants, the infectivity remained unimpaired. The subplants used for inoculation were incubated 7 to 9 days, were capable of inducing experimental poliomyelitis even when subcultured from tubes that had been stored in the incubator for 65 to 80 days. Parallel experiments in which heated cultures were used for inoculation into monkeys resulted negatively. Materials derived from culture tubes that contained no demonstrable organisms were also without effect. Control experiments included materials from normal monkeys and from the viruses of herpes, encephalitis, and neurovaccinia. All these, as well as the uninoculated culture medium by itself gave negative results.

Attempts to recultivate the typical organism from monkeys experimentally infected with the cultures and developing poliomyelitis, were successful with Berkefeld N filtrates and suspensions prepared from the nervous tissues of such animals.

The cycle of the disease has been demonstrated in monkeys inoculated with cultures. The organism has been developed from an invisible to a visible state and the typical disease induced in the experimental animal, in the brain and spinal cord of which the organism again returns to the invisible filterable stage.

It appears that the poliomyelitis virus has multiplied *in vitro*. The organisms cause pathological effects that can be augmented by successive passage of the culture material through a series of monkeys. Notable in this respect is the shortening of the incubation period.

The question of a possible life cycle and other biological considerations of the microbic agent are in the course of study. Additional inoculation experiments and investigations pertaining to morphology and virulence of cultures will be reported in the future.

5938

A Rapid Method for Determining Lactic Acid in Stools.

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In cultures, bacteria break down sugar into fatty acids, alcohol, lactic acid, methane, hydrogen and carbon dioxide. We have determined the amounts of the fatty acids occurring in human stools with various diets and under the conditions of constipation or catharsis.[1] Since lactic acid is produced in cultures in large amounts, this work was undertaken to answer the question whether lactic acid is present in stools in amounts comparable to those found for volatile fatty acids.

The isolation of lactic acid from stools could be carried out by extracting with ether or if a suitable precipitant were found which would free the filtrate from interfering substances, the filtrate might be directly oxidized. By means of the mercuric sulphate precipitation of West,[2] one can obtain from stool water clear filtrates practically free from nitrogen and almost free from interfering substances.[3] The filtrate from the mercury precipitation must be further treated by the usual copper sulphate and lime precipitation. Applying the method of Friedemann, Cotonio and Shaffer,[3] we found that the amounts of oxidizable material in such filtrates is small as shown by the fact that 100 cc. of filtrate, representing from 5 to 10 gm. of stool, require in the lactic acid apparatus less than 50 cc. of 0.01 N potassium permanganate to show a permanent excess of permanganate. As a matter of fact, there was obtained only 0.2 to 0.5 mg. of sulphite binding substance from the above mentioned amount of material.

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[1] Grove, E. W., Olmsted, W. H., and Koenig, Karl, *J. Biol. Chem.*, 1929, **85**, 127.

[2] West, E. S., Scharles, F. H., and Peterson, V. L., *J. Biol. Chem.*, 1929, **82**, 137.

To obtain filtrates that do not give a biuret test, we found it necessary to use 2 volumes of a 30% mercuric sulphate solution in 10% sulphuric acid to one volume of stool. The dilution of the precipitated stool was found to be important. Thus to one volume of stool was added 2 of mercuric sulphate solution. The mercury was precipitated with 10% alkali, usually 2 to 3 volumes being necessary and the final dilution with water was to 10 volumes. Table I shows that with this dilution the best return of added zinc lactate was obtained. Furthermore, this dilution made possible rapid filtration through ordinary filter paper.

The use of relatively large amounts of stool and the resulting larger amounts of mercuric sulphate solution determined our choice of the alkali used to precipitate the mercury salt. As suggested by West,[2] barium carbonate is the ideal alkali to precipitate mercury but the huge amount of this salt required to precipitate the volume of mercuric sulphate solution necessary in this work prohibited its use. For the same reason and especially because of the evolution

TABLE I.

Showing the Dilution of Precipitated Stool Influences the Recovery of Added Lactic Acid. 2 volumes of 30% mercuric sulphate solution to 1 volume of stool. Total volume 100 cc.

Amount of stool precipitated	Sulphite binding substance expressed as lactic acid	Added zinc lactate expressed as lactic acid	Total lactic acid found*	Recovery
Gm.	Mg.	Mg.	Mg.	%
5	.36	1.21	1.27	75
7	.45	1.21	1.50	87
10	.53	1.21	1.53	82
14	.68	1.21	1.52	71
20	.82	1.21	1.62	66

*Sulphite binding substances expressed as lactic acid.

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[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

of gas, sodium bicarbonate was not used. A 10% solution of sodium hydrate was found to be most satisfactory.

Of importance is the reaction of the stool during and at the end of precipitation. While the alkali is being added, the stool must be very vigorously stirred to prevent islands of strong alkali. For the same reason, the solution of sodium hydrate must be added slowly. The best recovery of added lactate was obtained when the reaction at the finish of the precipitation by alkali remained strongly acid to congo red paper. We suspect that the recovery of added lactate would have been better if it had been possible to use a weaker alkali.

Our method is as follows: To 100 gm. of stool, 200 cc. of a 30% solution of mercuric sulphate in 10% sulphuric acid was added. After passing through an ordinary kitchen sieve, 200 gm. portions of stool mixture were weighed out. From a burette, a 10% solution of sodium hydroxide was slowly added while stirring vigorously. At the end of the precipitation the reaction of stool was always just acid to congo red paper. The mercuric sulphate was added to stools immediately after defecation in order to sterilize them. Precipitation was done within a few hours, for if stools are allowed to stand in contact with the strongly acid mercuric sulphate solution a long time, the amount of sulphite binding substances is increased. The precipitated stool was made up to a liter and filtered. The filtrate was made more strongly acid by adding 1 cc. of 10% sulphuric acid. What mercury remained was precipitated by hydrogen sulphide. The excess gas was blown off with air saturated with moisture. After filtration, enough powdered copper sulphate was added to make a 2.5% solution[4] and calcium hydroxide was added until the solution was alkaline. After the alkaline copper precipitation had been allowed to stand in a refrigerator over night, the filtrate from the precipitation was oxidized by the method of Friedemann, Cotonio and Shaffer.[3]

100 cc. of filtrate was used for each determination. Determinations were made in duplicate and at the same time duplicate oxidations of pure solutions of zinc lactate were always run. The filtrate was neutralized by using phenol red as an indicator, after which 5 cc. of 2 M phosphoric acid and 10 cc. of 10% manganese sulphate were added. Oxidation was carried out with 0.01% potassium permanganate solution. We found, in agreement with Friedemann and Kendall,[5] that the optimum concentration of phosphoric acid is between 0.06 and 0.10 M.

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[4] **Ronzoni, Ethel, and Wallen-Lawrence, Zonja, *J. Biol. Chem.*, 1930, 74, 363.**

[5] **Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, 82, 23.**

TABLE II.

Date and Subject	Weight of Stool	Wt. of Fraction of Stool	Lactic Acid* in Fraction	Added Lactic Acid	Total Lactic Acid* Found	Recovery	Total Lactic Acid* in Stool
	gm.	gm.	mg.	mg.	mg.	%	mg.
2/11/30-P	78	3.12	.389	1.22	1.47	88	9.7
2/13/30-P	252	7.46	.432	1.23	1.57	92.3	14.5
2/17/30-P	171	5.76	.528	1.23	1.57	85	15.7
2/25/30-P	200	6.40	.731	1.24	1.72	80	22.8
2/20/30-P	234	7.14	.391	1.21	1.36	80	12.9
2/28/30-P	142	5.04	.179	1.21	1.28	91	5.04
3/ 4/30-P	105	3.98	.095	1.21	1.21	93	2.5
3/ 5/30-P	289	8.12	.383	1.21	1.36	81	13.6
3/13/30-P	116	6.25	.276	.605	.828	91	5.1
3/14/30-O	107	7.14	.528	1.21	1.51	81	22.5
3/17/30-P	110	5.00	.140	1.21	1.16	84	3.08
3/18/30-O	184	5.00	.320	1.21	1.38	88	11.8*
3/18/30-P	106	3.33	.185	1.21	1.16	81	5.95

*Sulphite binding substances expressed as lactic acid.

In our own stools while taking a mixed diet, we could find but a few milligrams of sulphite binding materials (Table II).

The recovery of zinc lactate added to these stools amounted to 80 or 90%. This is in agreement with the results of Cori and Buchwald,[6] who used the same method in recovering lactic acid from bodies of frogs. Recovery of lactic acid from solutions of pure zinc lactate were 96 ± 2%. In answer to the question, do small amounts of sulphite binding material arise from the oxidation of traces of lactic acid in stools, the following experimental facts should be considered. If one allows a stool to stand in contact with the acid mercuric sulphate solution for a period of several days, the amounts of sulphite binding substance will be doubled. Or if the stool filtrate is made alkaline and volatile substances are distilled off by steam, the sulphite binding substances are increased as much as 10 to 15%. However, if the stool filtrate is made acid and volatile acids distilled off, the sulphite binding substances are of the same value as before distillation. The data in Table I show that aliquot portions of stools when precipitated and oxidized do not give rise to aliquot amounts of sulphite binding materials. One can hardly conclude that we are dealing with sulphite binding substances arising from the oxidation of lactic acid alone.

Cori[7] has shown that lactic acid is absorbed at a much slower rate from the intestinal canal of the rat than similar amounts of the

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[6] Cori, Carl F., and Buchwald, K. W., *J. Biol. Chem.*, 1931, **92**, 367.

[7] Cori, G. T., *J. Biol. Chem.*, 1930, **87**, 13.

TABLE III.

	Sterilization	Wt. of Fraction of Stool	Lactic* Acid in Fraction	Added Lactic Acid	Total Lactic Acid* Found	Recovery
	min.	gm.	mg.	mg.	mg.	%
A. 50 gm. stool plus 129 mg. lactic acid	0	5	.17	12.9	.18	0.08
B. 50 gm. stool plus 129 mg. lactic acid	30	5	.17	12.9	10.73	82.00

*Sulphite binding substances expressed as lactic acid.

various sugars. It is with difficulty, however, that our results can be explained on the basis of complete absorption, for lactic acid has been found in the stools of infants.[8, 9] The obvious explanation is that the intestinal flora of the adult destroy lactic acid. This conclusion is confirmed by the incubation for 24 hours at 37°C. (Table III) of lactic acid added to stool before and after sterilization. The lactic acid added to a stool incubated for 24 hours disappears. The usual recovery was found when the lactic acid was incubated with a sterilized stool. Kendall, Friedemann and Ishikawa[10] have shown that bacteria in the resting state destroys lactic acid. This is particularly true of the colon bacillus group.

Conclusions. 1. A rapid method for determining lactic acid in stools is presented. 2. Only a few milligrams of sulphite binding material was found in the study of the stools of 2 normal adults. 3. Evidence is presented that lactic acid is destroyed by intestinal bacteria.

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Complement Fixation in Variola and Vaccinia.

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Certain writers have drawn attention to the application of the

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[8] Gerstley, J. R., Wang, C. C., and Wood, A. A., *Am. J. Dis. Child.*, 1930, **39**, 487.

[9] Gerstley, J. R., Wang, C. C., and Wood, A. A., *Am. J. Dis. Child.*, 1930, **39**, 729.

[10] Kendall, A. I., Friedemann, T. E., and Ishikawa, M., *J. Infect. Dis.*, 1930, **47**, 186.

complement fixation reaction in variola as a possible method in diagnosis. While in general the results have been irregular, they have, nevertheless, indicated that the reaction may be specific. Studying the serologic reactions of vaccinia, Bedson and Bland[1] have recently demonstrated that fixation conducted at room or ice box temperature is superior to that carried out at 37°C., the temperature apparently uniformly used heretofore. The work of Gordon,[2] elaborated by Burgess, Craigie and Tulloch,[3] and others, revealed a high degree of sensitivity and specificity in the flocculation test in variola, and showed the possibility of an *in vitro* diagnostic test.

To be of value, such a test must be applicable early in the disease. It must be sensitive and specific, and should require only a small amount of material.

The following technique was employed. Rabbits were hyper-immunized to vaccinia by intravenous and intraperitoneal injections of the neurovirus of Levaditi, previously prepared by testicular passage. The serum of these animals fixed complement with the homologous antigen in a dilution of 1 to 64. The well-known tendency of rabbit serum to anticomplementary action and non-specific fixation was avoided by using a 1 to 10 dilution. To reduce the volume sufficiently, the reagents were titrated by the drop method, using capillary pipettes, the same pipettes being used in the test. The final volume was about 0.3 cc. in tubes of 10x75 mm. Material collected from vesicles or pustules in capillary tubes was diluted with 2 drops of saline, half being used in the test and half serving as an anticomplementary control of the antigen. The usual reagent controls were included, and fixation carried out for 3 hours in the ice box.

Through the kindness of Dr. W. F. Wild, of the Health Depart-

TABLE I.

Source	No. of Cases	Time of Collection	Number Positive	Number Negative	Remarks
Vaccinia	7	6 to 10 days after primary vaccination	7	0	
Variola	16	1 to 14 days of eruption	14	2	Negative on 12th and 14th days. Decrustation almost complete.
Control	10		0	10	Varicella, impetigo, pemphigus, exfoliative dermatitis.

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[1] Bedson, S. P., and Bland, J. O. W., *Brit. J. Exp. Path.*, 1929, **10**, 393.

[2] Gordon, M. H., Med. Res. Council, Special Report Series, **98**.

[3] Burgess, W. L., Craigie, James, and Tulloch, W. J., Med. Res. Council, Special Report Series, **143**.

ment of Bridgeport, Connecticut, it was possible to obtain material from a number of cases of variola. This was studied as indicated above in the complement fixation reaction. The results obtained are summarized in Table I. The data show that material from all except 2 cases of variola gave complete fixation. In those not fixing complement, the material was obtained on the twelfth and fourteenth days of the eruption, when only a few pustules remained. There was no fixation by any of the material derived from a number of different conditions, and in no instance was the antigen anticomplementary.

On the basis of these observations it is suggested, therefore, that complement fixation may be of value in the early diagnosis of variola.

5940

Relation of the Lipids to Physiological Activity. Changes in Lipids of the Epidermis During Keratinization.*

D. J. KOOYMAN. (Introduced by D. P. Barr.)

From the Department of Dermatology, Washington University School of Medicine, and the Barnard Skin and Cancer Hospital, St. Louis.

Bloor[1] has shown relationships between physiological activity and the nature of the lipids in various tissues of the animal body. A high degree of activity is paralleled by a high phospholipid to cholesterol ratio. Since cells of the epidermis undergo a marked change in physiological activity during keratinization, we determined some of the lipids in epidermal cells before and after this process. The epidermis was separated from the cutis by digesting the skin, from which all subcutaneous fat had been removed, in cold 1% acetic acid. The completeness of the separation was confirmed by microscopic examination of sections of the skin. Several samples of epidermis were analyzed separately. Since it was impossible to obtain sufficiently large samples composed entirely of *stratum germinativum,* material containing considerable but varying quantities of this layer of active cells was scraped from the proximal surface of the epidermis and analyzed. Samples composed entirely of stratum corneum, trimmed from the soles of feet, were obtained from chiropodists and the lipids in keratinized cells determined. Skin from palms and soles of human cadavers was used because of the absence of hair and sebaceous glands and the greater thickness of the epidermis on these parts.

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* This work was made possible by a grant from the Lambert Chemical Company.

[1] Bloor, W. R., Okey, R., and Corner, G. W., *J. Biol. Chem.*, 1930, **86**, 291.

The material for analysis was cut into fine bits with scissors, or by passing it through a meat chopper, and extracted with hot alcohol vapors in a continuous extractor for 12 hours, changing the alcohol every 3 hours. The combined alcohol extracts were evaporated under diminished pressure and the residue extracted with ethyl ether. The ether extract was made up to volume in a volumetric flask and aliquots were used for the analyses.

The completeness of this extraction method was tested by saponifying several samples of extracted material with concentrated sodium hydroxide and extracting the remaining lipids with petroleum ether after acidification. In no case did the petroleum ether extract contain more than 4% of the total amount of lipid extracted from the material.

The phospholipid content was determined indirectly by the ether-soluble phosphorus, by the titration method of Widmark and Vahlquist,[2] and multiplying by a theoretical factor for lecithin. Bloor's oxidation method[3] was used for the determination of total fatty acids, and Okey's application of this to the digitonin method for cholesterol.[4]

The results in Table I are expressed as percent of the dry extracted skin.

TABLE I.

Material	No. of Samples	Phospholipid	Cholesterol	Fatty Acids	*Psl.* Chol.
Epidermis	8	Mean 0.62	0.90	1.71	0.70
Basal Layers	5	Mean 2.62	1.56	3.94	1.8
Str. Corneum	3	Mean 0.14	0.73	2.87	0.20

The basal layers of the epidermis have a lipid and phospholipid content comparable with that of the more active glandular tissues of the body. During the process of keratinization there is a diminution in all of the lipids, but the most striking loss occurs in the phospholipids, thus making the ratio of phospholipid to cholesterol much lower for the inactive keratinized cells than for the active cells of the basal layers. These results fit in very well with the concepts of Bloor concerning the lipids and physiological activity.

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[2] Widmark, G., and Vahlquist, B., *Biochem. Z.*, 1931, **230**, 245.

[3] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[4] Okey, Ruth, *J. Biol. Chem.*, 1930, **88**, 367.

5941

Peripheral Vaso-Constriction by Tobacco Demonstrated by Skin Temperature Changes.

WALTER G. MADDOCK and FREDERICK A. COLLER.
(Introduced by L. H. Newburgh.)

From the Department of Surgery, University of Michigan.

The skin temperature of normal individuals lying quietly, unclothed, on a stretcher in a room of constant temperature and air currents reaches a reliable constancy at a given body point in about one hour. Thereafter, variations in skin temperature are due to changes in vaso-constrictor tonus. Variations tend to occur more commonly and with greater amplitude towards the tips of the extremities. Measurements of the magnitude of such variations can have a definite value in well controlled psychological, physiological and pharmacological experiments.

Our interest in tobacco smoking arose as a result of its alleged etiological relationship to peripheral vascular disturbances. An example of our investigation is shown in Fig. 1. Subject K. H.

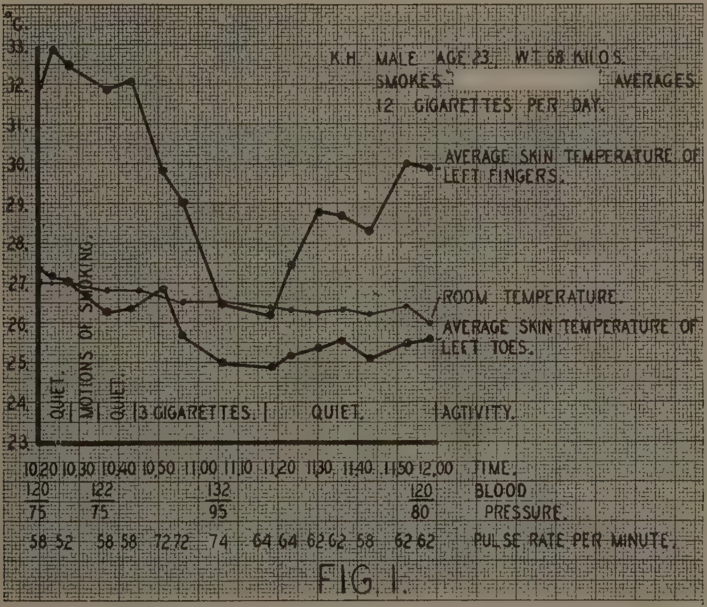

Fig. 1.

Readings began one hour after exposure to environmental conditions. The results show: 1. A rise in blood pressure and pulse rate while smoking, followed by a gradual return to the previous level on cessation of smoking. 2. A fall in the average skin temperature of the left fingers from 32.1°C. to 26.2°C., and in left toes from 26.4°C. to 24.9°C. while smoking, followed by a gradual rise in the skin temperature on cessation of smoking. A negligible change in the skin temperature occurred while going through the motions of smoking with a small paper tube.

This peripheral vaso-constrictor effect of tobacco smoking was demonstrated in man by Bruce, Miller and Hooker,[1] and Simici and Marcu,[2] using the volume changes in the hand and arm.

In general, the results shown with K. H. have been corroborated in 20 other subjects, both light and heavy smokers. Control experiments substituting cubebs for cigarettes, using the water pipe principle or first passing the smoke through a layer of cotton moistened with $FeCl_3$, produces a negligible fall in the skin temperatures. These controls substantiate our opinion that the peripheral vaso-constriction produced is the result of active products absorbed from the tobacco smoke.

5942

Influence of Vitamin A and B Deficiency upon Intestinal Acid-Base Equilibrium and Bacterial Flora.

E. E. SEIDMON AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

The intra-intestinal acid-base equilibrium and the distribution of the bacterial flora have been investigated in certain vitamin deficiencies. The basic diet consisted of casein, cornstarch, Osborne and Mendel salt mixture, butter fat and dried yeast. In the vitamin A deficient diet, the butter fat was omitted. In the vitamin B deficient diet the yeast was omitted. Sixty-five 50 gm. rats were placed upon each of these 3 diets. The vitamin A deficiency series were killed in gas chamber after 7-8 weeks, the B deficiency series after 3-4 weeks. The end point was determined at the time the weight curve was stationary for 4 successive days. The controls were fed for 8 weeks.

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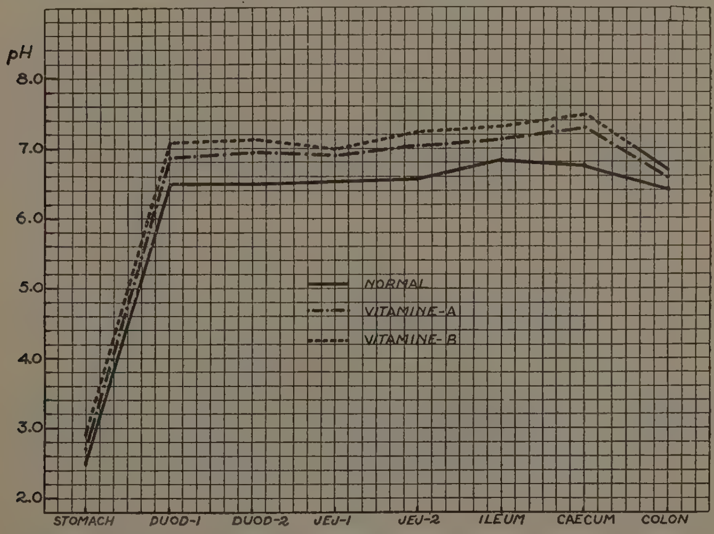
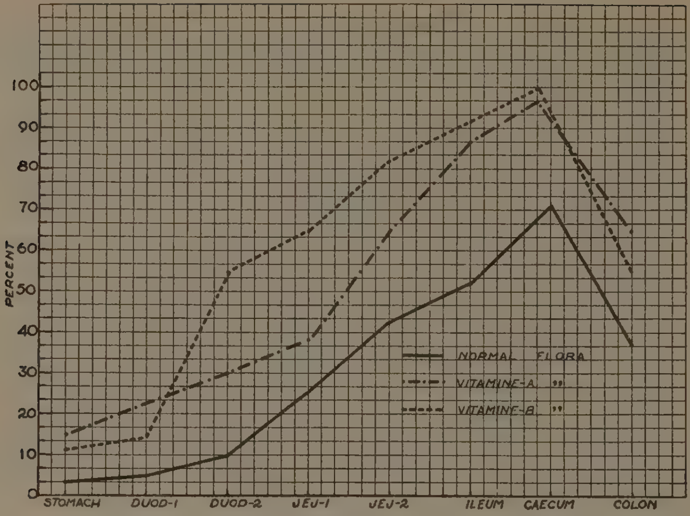
[1] Bruce, James W., Miller, James R., and Hooker, Donald R., *Am. J. Physiol.*, 1909, **24**, 104.

[2] Simici, D., and Marcu, I., *J. de Physiol. et de Path. Gen.*, 1927, **25**, 58.

All animals were put in single cages and fasted for 24 hours. Illuminating gas lethal chambers were used to kill the rats. The specimen of gastro-intestinal contents were removed after ligature of the segments with hemostats. The upper portion of the tract was empty and to make conditions comparable, 0.4 cc. of distilled water was used to flush out each 3 cm. length segment of the isolated loop to obtain specimen. Finely drawn out Wright's pipettes were used. One drop was used to seed surface of agar plates, the same spreader was used for a second plate for dilution. The capillary Heiligs colorimeter was used to determine the h-ion concentration of the same specimen. Not more than 5 minutes must elapse before this determination is made, otherwise the pH becomes progressively more alkaline with time until the blood reaction is reached. The number of colonies have been recorded. We can best express our results by taking the caecal bacterial flora of the highest count as 100 and arranging the various segments in percent of these figures. The



GRAPH 1.
Ordinate: pH of the contents of the gastro-intestinal contents.
Abscissa: various segments of the gastro-intestinal tract.
Continuous line represents the normal. Dot-dash line represents the vitamin A deficiency series. Broken line represents the vitamin B deficiency series.



GRAPH 2.

Ordinate: % of bacterial flora, the caecal flora of the vitamin B series was the heaviest and is taken as 100.

Abscissa: segments of gastro-intestinal tract as indicated.

The lines indicating the same as in Graph I.

vitamin deficient rats had a denser bacterial population within the intestinal lumen than the normal. The H-ion concentration also shows a difference. The accompanying graphs show the results in condensed form. Each line represents the average findings of 65 rats.

5943

Absorption of Yeast from the Large Intestine.

VIRGINIA FISHER. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

Following investigations into absorption of yeast from the small intestine,[1] a series of experiments have been conducted to demonstrate absorption of yeast through the wall of the large intestine into the splanchnic circulation.

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[1] Fisher, Virginia, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 948.

A soft rubber tube (vaselined) was inserted into the rectum of a dog so that the tip was 35 cm. from the anus. Yeast in doses varying from 8 to 31 billion cells suspended in 20 cc. of normal saline was injected by means of a syringe attached to the end of the tube. The animals were killed at 15, 30, and 60 minute intervals. Material from the various segments of the gastro-intestinal tract was obtained upon swabs and cultured on 4% maltose acid agar. One gram specimens of the liver, lymph, lung, spleen, and kidney were removed, macerated in 10 cc. of 4% maltose acid broth. One tenth cm. was cultured on maltose agar immediately; and again after 24 hours' incubation, on the same medium.

Arnold and Nedzel[2] have demonstrated experimentally that egg-white increased absorption of bacteria into the splanchnic circulation. As yeast was not absorbed in appreciable quantities in the above experiments, a second series of 16 dogs were inoculated using eggwhite to increase, if possible, the number of yeast cells absorbed. The same technic was used except that the yeast cells were mixed with the whites of 2 eggs strained through cotton to remove the undissolved strands.

A third series of dogs were anesthetized, the abdomen opened and the gastro-intestinal tract ligated at the ileo-caecal valve. Twenty cubic cm. of eggwhite was given by mouth and 20 cc. of a yeast suspension by rectum. The procedure followed thereafter was the same as above.

TABLE I. *Typical Protocols.*

Dog No.		Cultures	Liver	Lymph	Lung	Spleen	Kidney
	15 minutes						
I	Control	Immed.	1600	—	1	—	—
		24 hr.	1500	—	9700	—	—
II	Eggwhite (rectum)	Immed.	200	—	—	—	500
		24 hr.	900	5400	2100	1400	36,300
III	Eggwhite (mouth)	Immed.	33	—	—	—	—
		24 hr.	2300	36,000	30,000	—	93,000
	30 minutes						
I	Control	Immed.	500	300	700	—	300
		24 hr.	300	—	—	—	—
II	Eggwhite (rectum)	Immed.	200	1800	800	700	300
		24 hr.	6000	12,000	27,000	2300	7200
III	Eggwhite (mouth)	Immed.	100	300	900	200	150
		24 hr.	58,100	360,000	144,000	7200	57,000
	60 minutes						
I	Control	Immed.	100	100	—	—	100
		24 hr.	—	—	100	400	—
II	Eggwhite (rectum)	Immed.	100	—	100	—	—
		24 hr.	300	200	10,000	700	1600

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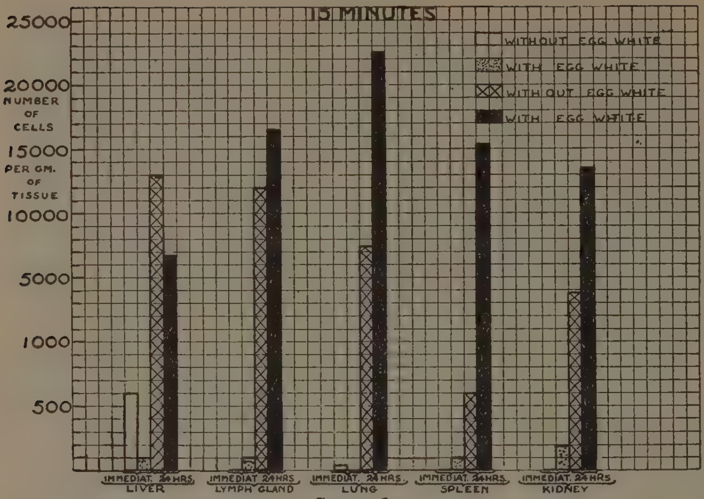
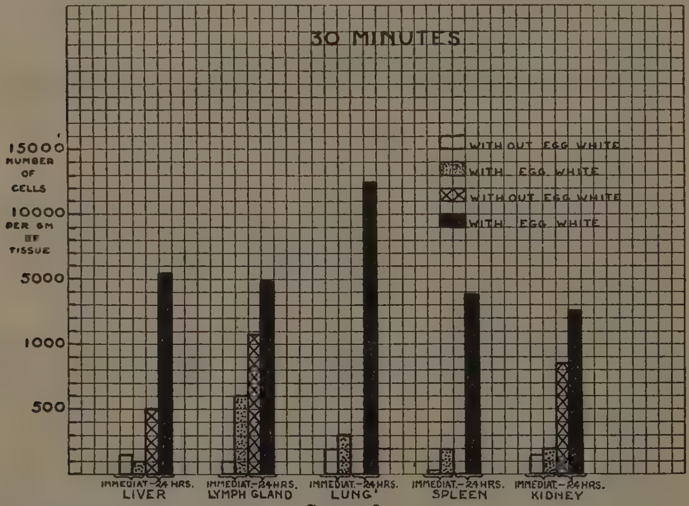
[2] Nedzel, A. J., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 358, 360, 361, 364, and 366.



GRAPH 1.

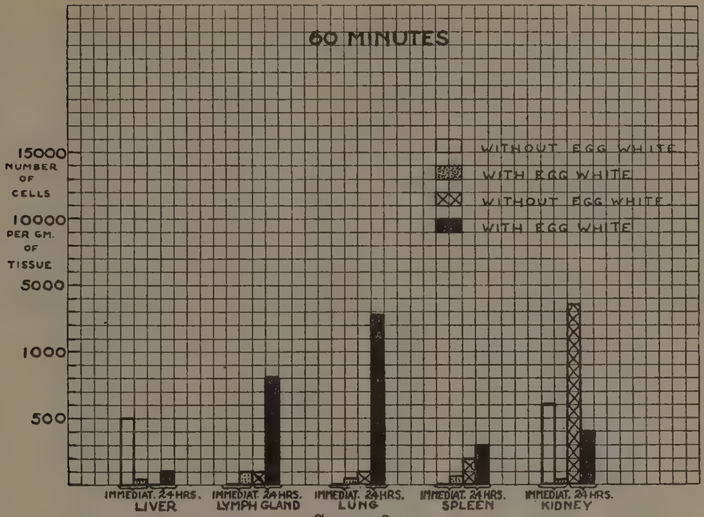
Distribution of viable yeast in organs of dogs given yeast by rectum and killed 15 minutes after injection.

Plain column represents number of yeast in immediate cultures (yeast suspended in saline). Dotted column represents number of yeast in immediate cultures (yeast suspended in eggwhite). Crossed column represents number of yeast in 24-hour cultures (yeast suspended in saline). Solid column represents number of yeast in 24-hour cultures (yeast suspended in eggwhite).



GRAPH 2.

Distribution of viable yeast in organs of dogs given yeast by rectum and killed 30 minutes after injection.



Graph 3.

Distribution of viable yeast in organs of dogs given yeast by rectum and killed 60 minutes after injection.

Table I gives typical protocols from each of the experiments described above. Graphs 1, 2, and 3 present results on controls and those in which eggwhite was mixed with the yeast.

In the control series, a group of animals were anesthetized, the abdomen opened, and the gastro-intestinal tract ligated at the ileocaecal valve. Increased absorption in these instances may be due to trauma.

The mechanism of the action of eggwhite is not clearly understood. Graphs 1, 2, and 3, except in 2 instances demonstrate that absorption from the large intestine is increased when the yeast is suspended in eggwhite. Since animals given yeast by rectum and eggwhite by mouth also show increased absorption over control animals, our results indicate that one of the actions of eggwhite may be due to inhibition of killing power of the body for yeast.

One incidental finding is of interest; a bitch 4 to 6 weeks pregnant in the control series, showed absorption of yeast from the rectum in numbers comparable to those animals given eggwhite.

Some of the immediate cultures show more yeast than do the 24-hour cultures. This is probably due to the toxic products within the organ which are liberated when the tissue is macerated and allowed to incubate in broth. This reaction seldom occurs when eggwhite is used.

The largest amount of absorption occurs at 15 and 30 minutes and decreases therefrom; 2 dogs killed at 2 hours after injection were negative throughout.

Conclusions. 1. Yeast is absorbed from the lumen of the colon in greatest numbers at 15 minutes, diminishing thereafter until none can be demonstrated at the 2-hour interval. 2. Eggwhite mixed with yeast increases the number of yeasts absorbed from the rectum of the dog. 3. A greater number of viable yeast cells can be demonstrated to be present in certain organs after the application of eggwhite to the duodenal mucosa and yeast introduced into the lumen of the rectum. This may not be due to increased absorption but to a decrease in the destruction of yeast in the body.

5944

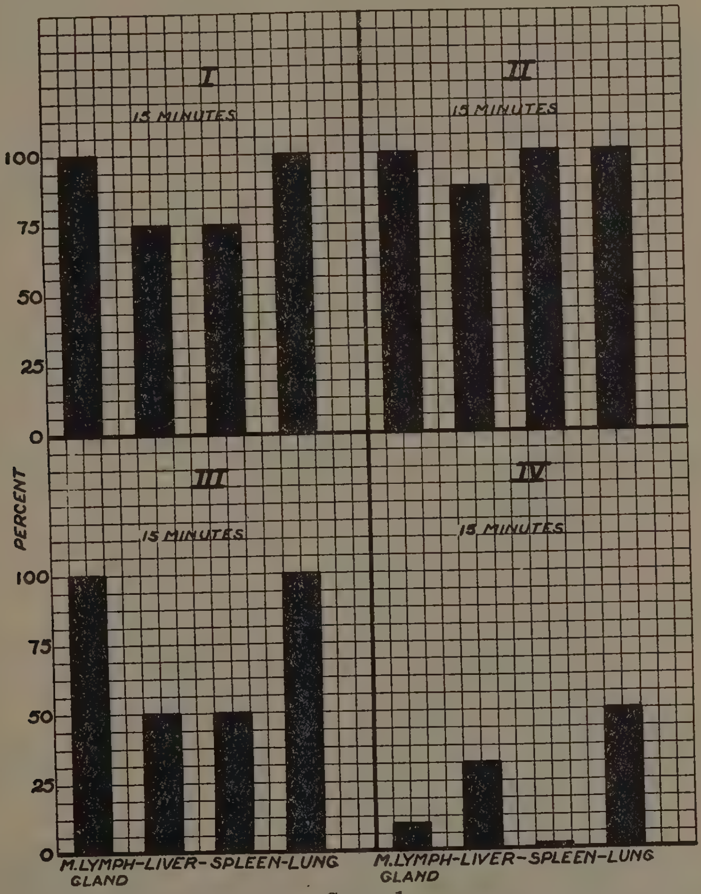
Absorption of Bacteria from the Large Intestine.

M. SHUGER AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

B. prodigiosus, B. murii, and *B. welchii* have been placed in the lumen of the large intestine of dogs. The animals were killed 15, 30, 45 minutes, 1, 2, 3, 4, and 24 hours after rectal injection and the mesenteric lymph node, liver, spleen and lung specimens were removed under aseptic precautions. The tissue was cut up with sterile scissors, placed in broth cultures and further macerated with glass rods. All cultures were incubated 24 hours. Subcultures were made on plain agar for *B. prodigiosus,* Endo media for *B. murii* and *B. coli;* dextrose broth, dextrose agar shake tubes and brain media were used to subculture *B. welchii.* Ether, chloratone and nembutal anesthetics were used. No differences in bacterial permeability were noted with different anesthetics. In some animals the large intestine was washed out with saline and in the beginning of our work we exposed the large intestine by abdominal incision. Anesthetics were used throughout to make our results comparable and avoid struggling of animals during experiment. Only a part of our experiments will be reported here.

Animals killed 15 minutes after rectal injection show the highest percent of positive organ cultures. One hour after rectal injection all organs are free of viable bacteria. The 30 and 45 minute intervals after rectal injection show progressively few bacteria in the organs cultured as compared to the 15 minute time interval. Sixteen dogs were killed and organ cultures made with same technic as



Graph 1.

Ordinate: % of animals showing viable bacteria.

Abscissa: the 4 organs cultured, mesenteric lymph node, liver, spleen, and lung.

I. Catheter introduced into rectum and 10 cc. of sterile saline injected. Ten dogs used, killed 15 minutes after injection. *B. coli* in organs recorded.

II. Same technic except *B. prodigiosus* injected in the 10 cc. of saline. The *B. prodigiosus* in organs recorded.

III. Same technic, except *B. prodigiosus* was suspended in alkaline phosphate buffer solution (pH 8.0). *B. prodigiosus* in organs recorded.

IV. Same technic, except *B. prodigiosus* was suspended in acid phosphate buffered solution (pH 5.0). *B. prodigiosus* in organs recorded.

controls. Two showed *B. coli* in the mesenteric lymph gland and this bacillus was isolated from the liver of one dog. Cocci groups—mostly enterococci—were cultured from the majority of mesenteric lymph nodes and liver of all dogs.

Mechanical irritation due to the introduction of a vaselined catheter and 10 cc. of sterile saline causes the appearance of viable *B. coli* in the internal organs if cultured within 15 minutes. After 45 minutes *B. coli* cannot be cultured from these same organs. We could not demonstrate any difference in permeability between *B. prodigiosus, B. murii,* and *B. welchii.*

The accompanying graph represents the 15 minute time interval after mechanical manipulation only, after *B. prodigiosus* in saline and after *B. prodigiosus* in alkaline and acid phosphate buffered solutions. The differences between the latter 2 solutions are very striking. Rats have also been used and the observations made upon dogs have been substantiated. The same technic and the same test bacteria were used. Fifteen minutes after mechanical manipulation associated with the introduction of a thin, oiled catheter through the anus and injection of 10 cc. of saline, viable bacteria can be demonstrated within certain organs. Endogenous bacteria are present when sterile saline is used, exogenous bacteria are present if they were suspended in the saline.

5945

Influence of Saponin upon Bacterial Flora, H-ion Concentration and Permeability of the Intestinal Tract.

A. J. NEDZEL, ROSS STONECIPHER AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

Kaufman[1] showed that the oral ingestion of small doses of saponin caused a sudden alteration in the bacterial flora and the acid-base equilibrium of the contents of the stomach and small intestine of mice. His work was part of a study upon the increased susceptibility of rodents to enteritides infections. We have extended Kaufman's experiments to dogs. In addition to the intra-intestinal changes we have studied the distribution of *B. coli* in the organs of these animals.

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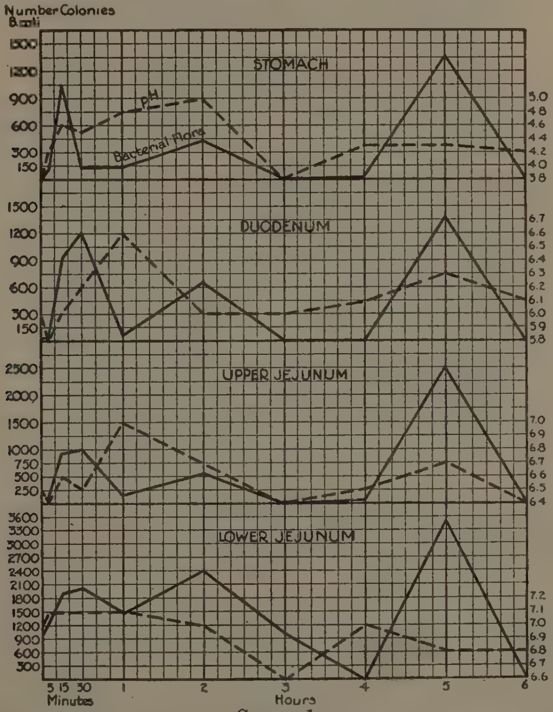
[1] Kaufman, I., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 21.

Thirty-four dogs were used. Two cm. of a 20% aqueous solution of saponin was administered by stomach tube. The animals were killed at various time intervals. The hydrogen-ion concentration of the contents of the stomach, duodenum, upper and lower jejunum was determined by Heilig's capillary comparator, and the distribution of *B. coli* was obtained by culture upon Endo media. The lymph glands of the splanchnic region, liver, kidney, spleen and lungs were also examined for the presence of *B. coli* by taking pieces from these organs, putting them into flasks of broth for 18 hours, then spreading 5 drops from these broth cultures on Endo media plates. The plates were read in 48 hours. No bacteria were administered in these experiments. The *B. coli* cultured in organs were endogenous strains.



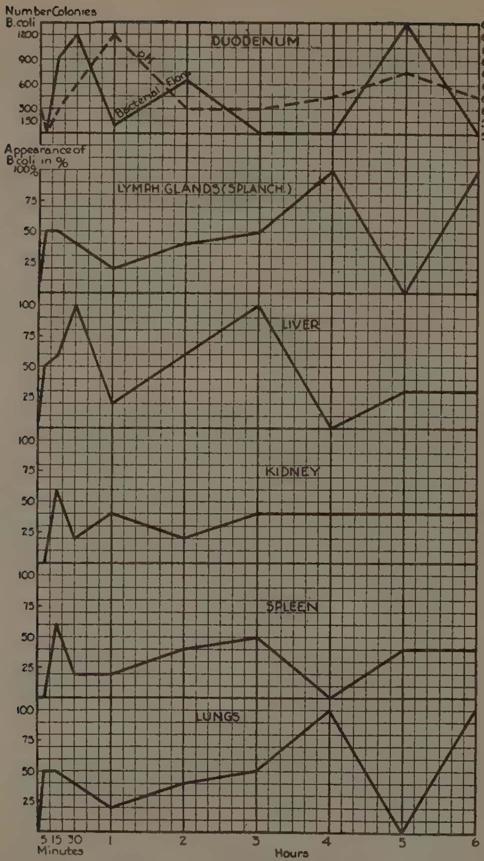
GRAPH 1.

Ordinate: left, number per swab of *B. coli* grown from contents of intestinal segments as indicated. Right, pH of same contents.

Abscissa: time as indicated.

The broken line represents H-ion concentration curve. The solid line represents *B. coli* count.

Two cubic centimeters of 20% saponin put in stomach at beginning of experiment.



GRAPH 2.

Ordinate: % of animals showing positive *B. coli* culture of organs indicated.
Abscissa: time as indicated.
The upper (duodenum) curve is reproduced from Graph 1 to indicate relationship of the intra-intestinal contents and organ cultures.

Graph 1 shows that saponin causes a change within the acid-base balance and the bacterial flora in dogs similar to those reported by Kaufman for rats. Graph 2 shows that there is associated with the intra-intestinal changes the sudden appearance within certain organs, mesenteric lymph node, liver, kidney, spleen, and lungs of viable *B. coli*.

The oral ingestion of saponin causes a sudden change in the acid-base equilibrium and in the endogenous bacterial flora. This is associated with the appearance of viable *B. coli* in the organs of these animals. The wave-like cycle of these chemical and bacterial changes reported here are characteristic of autonomic nervous system disturbances.

5946

Influence of Gastric Acidity upon Viability of Bacteria in Isolated Stomach.

A. J. NEDZEL and LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

Johnson and Arnold[1] in a series of experiments on dogs with a non-leaking gastric fistula have found that exogenous bacteria (*B. prodigiosus*) lose their viability in the presence of free hydrochloric acid and when the gastric contents were alkalinized with Na_2HPO_4 buffer solution these previously non-viable exogenous bacteria regained their viability. This phenomenon was so constant that we decided to set up acute experiments in which we could entirely separate the stomach from the duodenum. By this technic we could completely exclude the possibility of any regurgitation from the intestine.

The experiments on dogs were conducted in the following manner. The abdomen of an animal, under nembutal anesthesia (1 cc. of 3% solution per kilo intraperitoneally), was opened and the pyloric part of the stomach with the duodenum brought up to the surface. A series of ligatures was put around the pyloric end of the duodenum through the wall of the duodenum to avoid a hemorrhage into the stomach and then the duodenum was severed about 1 cm. below the ligatures. In such a manner we produced a wide and non-bleeding opening into the stomach which facilitated the securing of specimens from the gastric contents. All the specimens were taken by sterile Wright pipettes.

First, we determined the pH (LaMotte hydrogen-ion testing set) of the gastric contents, then introduced into the stomach a suspension of *B. prodigiosus* (washings of a 24-hour growth on agar plate in 20 cc. sodium chloride solution) and simultaneously injected 1.5 mg. of a histamine intravenously. At certain time intervals (see chart) a specimen from the stomach was taken and tested for pH and for the presence of viable *B. prodigiosus* (one drop of the stomach contents spread on an agar plate, readings in 24 hours). At the same time slides were made from the stomach specimens and stained by the Gram method. The slides were always positive for the presence of Gram negative bacillus. Ten dogs were used, averaging 35 pounds in weight. The stomach of each dog reacted differently depending on the functional status of the stomach. The results can be divided into 3 groups, I and III representing the average of 3 dogs, and II, 4 dogs.

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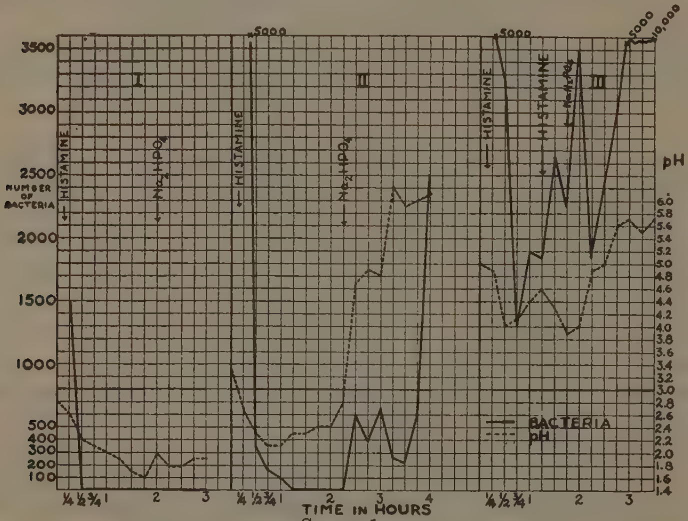
[1] Johnson and Arnold, Proc. Soc. Exp. Biol. and Med., 1931, **29**.



GRAPH 1.

Ordinate: Left, number of viable *B. prodigiosus* per cc. of gastric contents. Right, pH of gastric contents.

Abscissa: time in hours or fraction of hours.

Solid line represents the curve of viable bacteria in gastric contents. Broken line represents the pH of the gastric contents.

Group I (Chart I). The stomach contained free hydrochloric acid. The injection of histamine increased the concentration of acid. Na_2HPO_4 buffer solution (20 cc.) was introduced into the gastric lumen; no change in the h-ion concentration could be demonstrated. Numerous Gram negative bacilli were present in smears; were non-viable on nutrient agar.

In the second group (Chart II) where there was no free hydrochloric acid, the histamine stimulated acid secretion and the *B. prodigiosus* became non-viable. But the addition of Na_2HPO_4 buffer solution brought the pH up to 4.7 and higher. Simultaneously with this change toward acid-deficit the *B. prodigiosus* again became viable, as is clearly shown on the chart (II).

In the third group (Chart III) the gastric mucosa was less active. In this case the injection of the same dose of histamine did not cause the stomach to produce the free acid. The addition of Na_2HPO_4 buffer solution was followed by an increase in the number of viable bacteria. The introduced exogenous bacteria (*B. prodigiosus* in our case) remained viable throughout the experiment.

5947

Has the Free Gastric Acidity Bactericidal or Bacteriostatic Power?

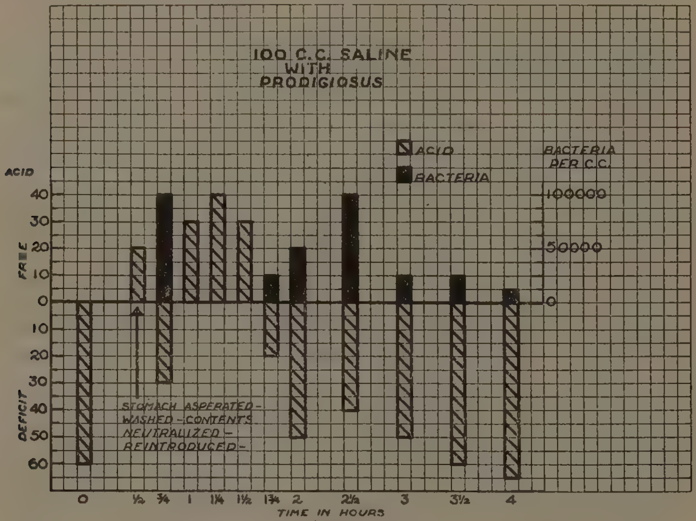
TITUS M. JOHNSON AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, College of Medicine, and Research Laboratories of the Illinois Department of Public Health, Chicago.

Viable bacteria were introduced into the stomach of a series of non-leaking gastric fistula dogs and cultures made at certain time intervals along with titrations of the free acid and acid-deficit. One agar plate of *B. prodigiosus* growth was washed off in 100 cc. of sterile saline. All animals were fasted 20 to 24 hours before experiments. When 100 cc. of a heavy *B. prodigiosus* suspension was placed in a gastric lumen containing free acid secretion, the subsequent specimens removed and cultured upon nutrient agar were sterile. If there was an absence of free acid in the stomach and the same procedure was followed, the nutrient agar plates were so overgrown that dilutions had to be made to estimate the bacterial content. Some experiments were carried out over 3 and 4 hours of time. It was observed that so long as free H-ions were present no viable bacteria could be demonstrated. But if an acid-deficit developed during the course of the experiment, we observed a sudden appearance within the lumen of the stomach of many viable *B. prodigiosus* and they persisted so long as free acid was absent. Test bacteria were only introduced at the beginning of the experiment, their presence or absence within the lumen of the stomach determined by subculturing on nutrient agar, could be correlated with the acid-base balance existing at the time within the cavity of this organ.

Over 300 experiments have been carried out, *B. prodigiosus, B.*

coli, and *B. murii* have been used as test bacteria. The results are the same with each bacteria. The test bacteria have been suspended in saline, milk, phosphate buffered solutions, and meat. Graph 1 illustrates an example where there was an acid-deficit at the beginning of the experiment and it persisted for one hour. Then free acid appeared. One hundred fifty thousand bacteria per cc. of gastric contents were present in a viable condition during the period of acid-deficit. As soon as free acid was detected no viable bacteria could be demonstrated. Sterile alkaline phosphate solution was introduced to neutralize the free acid. The original test bacteria reappeared and persisted during the period of acid-deficit. Graph 2 shows an experiment where saline and *B. prodigiosus* were introduced into an acid-deficit gastric lumen. Free acid appeared within a half hour; no viable bacteria could be demonstrated. The gastric contents were then removed through the fistula, cultured, neutralized with sodium hydroxide, again cultured, then replaced within the lumen of a second dog that had not been used for 2 weeks and whose stomach contained free acid. The neutralized gastric contents did

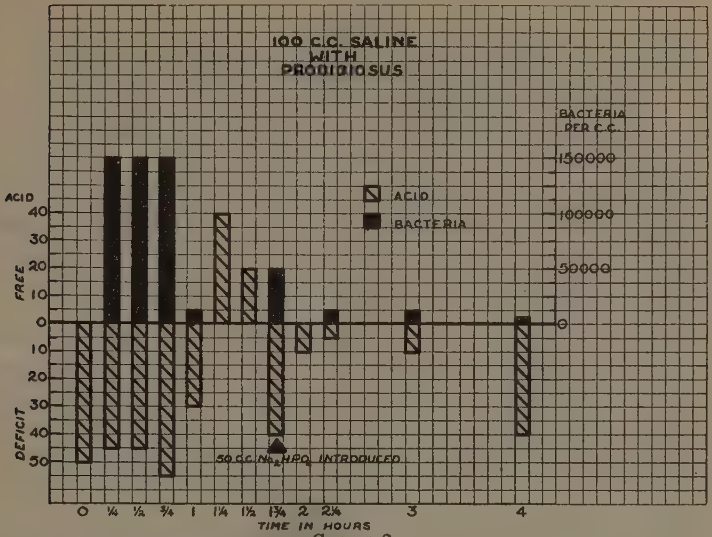


GRAPH 1.

Ordinate: Left, free and acid-deficit in clinical units in gastric contents. Right, Number of bacteria per cc. of gastric contents.

Abscissa: time in hours or fraction of hour from the beginning of the experiment.

One agar plate of *B. prodigiosus* suspended in 100 cc. saline was introduced into the stomach through fistula at the beginning of the experiment. Fifty cc. of sterile alkaline phosphate buffered solution introduced after one hour and 45 minutes at the time of free gastric acidity.



Graph 2.

Ordinate: Left, gastric acidity expressed in clinical units. Right, bacteria per cc. of gastric contents.

Abscissa: time in hours.

One agar plate of *B. prodigiosus* suspended in 100 cc. saline and introduced through fistula into the stomach. After free acid appeared (½ hour) the stomach was emptied. Cultures proved the absence of viable *B. prodigiosus*. The gastric contents were neutralized and introduced into the stomach of a second dog. Cultures again showed no viable *B. prodigiosus* present. Specimen removed from stomach showed heavy growths of *B. prodigiosus* during period of acid deficit.

not contain viable bacteria. One hundred thousand viable bacteria could be demonstrated per cc. of the gastric contents in the second dog during the initial period of acid-deficit. When free acid appeared, the contents were again sterile when put on agar. When an acid-deficit developed (after 1¾ hours) the original test bacteria reappeared in viable form. In all of our experiments many stainable bacteria were present in the gastric contents which resembled the test bacteria. We could not demonstrate any difference in relative numbers of stainable bacteria in the gastric contents of free acid and acid-deficit animals. Berkefeld filtration of the gastric contents apparently removes the bacteria; we were unable to demonstrate them in experiments similar to that in Graph 2, where the gastric contents were filtered before introducing into the second dog's stomach.

5948

Studies of the Effect of Atropine on Gastric Secretion.

ROBERT T. PORTER. (Introduced by W. L. Palmer.)
From the Department of Medicine, University of Chicago.

The purpose of the experiments reported in this paper was to determine the effect of small doses of atropine on the total volume of gastric secretion and its content of titratable acidity as these occurred in response to a standard injection of histamine hydrochloride in man.

Keeton, Luckhardt and Koch[1] studied the effects of atropine and histamine on gastric secretion in the dog. They found that the first effect of atropine is to decrease both the total quantity of gastric secretion and the pepsin content, whereas the acid concentration is not decreased until a sufficient amount of atropine has been given to reduce the total quantity of gastric secretion to an amount approximately 20 to 50% of its original value.

Keefer and Bloomfield[2] studied the effect of the subcutaneous injection of 2 mg. of atropine upon gastric secretion in man in 1926, using 50 cc. of 7% alcohol as the test stimulus instead of histamine. They reported a marked diminution in the total quantity of secretion and also a reduction in the acidity, but no parallelism in the degree of reduction was noted.

Polland[3] reported that in man atropine dissociates the normal response to histamine, producing a marked decrease in the volume of secretion and in the concentration and total output of pepsin, but he found a *rise* in the titratable acidity. He attributed this to the relatively slight inhibitory effect of atropine upon the total output of acid with a consequent increase in the amount of acid per cc. of gastric content.

In this work the following procedure was used: The tests were conducted in the morning before breakfast. The test stimulus used was 0.01 mg. of histamine hydrochloride per kilo body weight injected subcutaneously as recommended by Bloomfield and Polland.[4] The stomach was aspirated completely by means of a Rehfuss tube at 10 minute intervals until the secretion returned to normal, which it usually did within 60 minutes. The patient was warned not to

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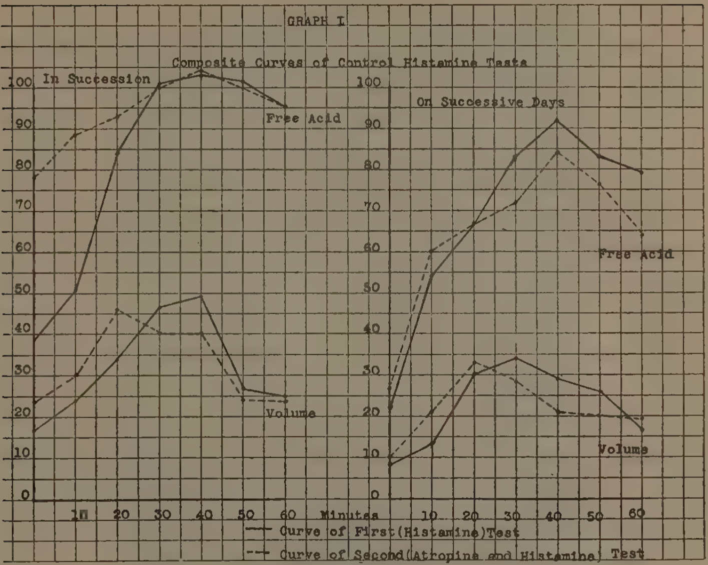
[1] Keeton, R. W., Luckhardt, A. B., and Koch, F. C., *Am. J. Physiol.*, 1930, **51**, 469.

[2] Keefer, Chester S., and Bloomfield, Arthur L., *Arch. Int. Med.*, 1926, **38**, 303.

[3] Polland, W. S., *J. Clin. Invest.*, 1930, **9**, 319.

[4] Bloomfield, Arthur L., and Polland, W. S., *J. Am. Med. Assn.*, 1929, **92**, 1508.



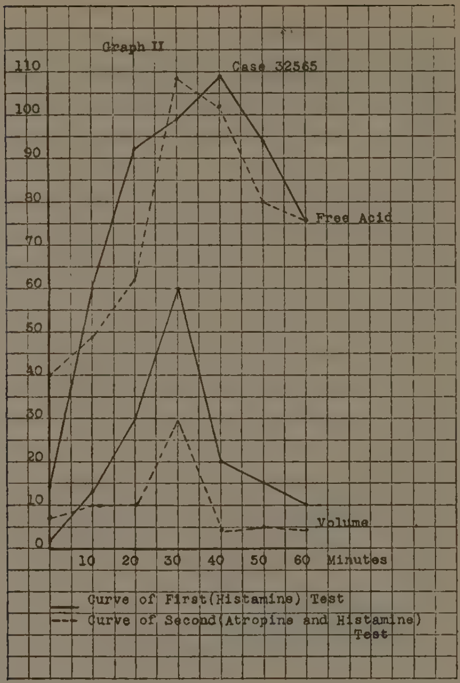
GRAPH 1.

swallow saliva during the test. In 4 control cases the test was repeated after about 90 minutes and in 4 other control cases it was repeated on the following day. In both cases the second curve was practically identical with the first. The first graph shows the composite curves of the control histamine tests; first, those run on the same day; and, second, those run on successive days.

The results from both methods were the same, showing that 2 histamine tests could be run on the same individual on the same or on successive days and give results with a variation of not more than 5 to 10%. This is in accord with the findings of Polland.[3]

The atropine experiments were carried out on 20 patients. In 8 of them a histamine test was first given and the gastric secretion allowed to return to normal. A subcutaneous injection of 1 mg. of atropine was then made, followed 15 minutes later by a second histamine injection. In 12 of the reported cases, 24 hours were allowed to elapse between the 2 tests. They are reported together because the results were the same.

The results of the tests, as shown in Graph 2, which is the chart of a single atropine test, and in Graph 3, which is the chart of the combined results of the 20 atropine tests, show that the height reached by the free acidity (Topfer's Reagent) is not affected by

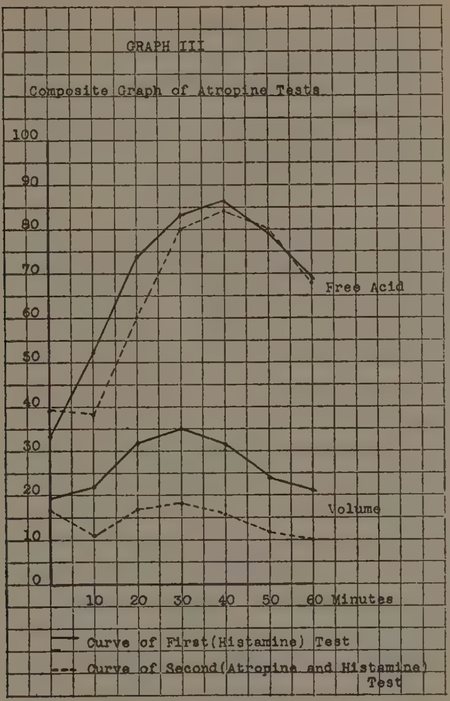


GRAPH 2.

atropine in the dosage used but the volume is quite definitely decreased, the reduction varying from 40 to 60% with an average reduction for the 20 tests of 51%.

These findings are quite in accord with those of Keeton, Luckhardt and Koch in the dog, and of Keefer and Bloomfield in man, but differ from those of Polland in that the latter reported a rise in the titratable acidity after atropine. This discrepancy is probably not due to a difference in the amount of atropine used, 1 mg. in these experiments as compared with 0.2 mg. per 10 kg. body weight in those of Polland. It may be due to the fact that a longer interval was allowed to elapse between the tests, 90 to 120 minutes in this series as compared with 50 to 60 minutes in that of Polland. The findings, however, are compatible with Polland's suggestion that water and acid may have different mechanisms of secretion.

Conclusions. 1. Gastric secretion obtained by histamine stimulation (0.01 mg. per kilo) under standard conditions, seems to be quite constant for the same individual, both as to volume and height



Graph 3.

of free acidity. 2. Histamine tests repeated in succession and on succeeding days give practically identical results. 3. Atropine, in doses of 1 mg., decreases the volume of gastric secretion obtained by histamine stimulation in man, approximately 50%, but does not affect the free acid content appreciably.

5949

Colloid Osmotic Pressure of Muscle.

P. A. DUFF. (Introduced by E. F. Adolph.)

From the Department of Physiology, School of Medicine and Dentistry, the University of Rochester.

Measurements were made of the negative pressure necessary to prevent the passage of fluid across prepared cellophane membranes from blood serum into the cut surface of muscle. The membranes were conditioned with 95% alcohol and tested for their permeability to NaCl, glucose, and egg albumin. Membranes were used which would allow an equilibrium of non-colloids to be established between muscle and serum in 24 hours or less. A piece of the prepared membrane was cemented tightly across the mouth of a small glass osmometer with shellac and collodion. From 0.04 to 0.1 cc. of blood serum was introduced into the osmometer with a capillary pipette through the stopcock which was used to seal off the contents of the osmometer. Transverse sections of dog or cat muscle were tied tightly to the mouth of the osmometer so that the cut surface of the muscle entirely covered the surface of the membrane. The whole was enclosed in a glass chamber immersed in a regulated water bath.

In 6 measurements, negative pressures from 18 to 33 mm. Hg. were found necessary to prevent the passage of fluid from serum into muscle. The colloid osmotic pressures of the serums used in these experiments were also measured in the same apparatus, and ranged from 13 to 24 mm. Hg. Adding the colloid osmotic pressure of the serum vs. Ringer to the negative pressure measured with the serum vs. muscle, values were obtained ranging from 37 to 48 mm. Hg. The colloid osmotic pressure of the contents of muscle cells is believed to lie in this range. This pressure is probably not effective *in vivo*, being balanced by other forces such as intracellular turgor pressure.

5950

Influence of Ortho-Substitution on the Conjugation of Benzoic Acid.

ARMAND J. QUICK.

From the Department of Surgical Research, Cornell University Medical College.

Substitution of benzoic acid in ortho position almost completely inhibits in the dog the conjugation with glycine. The effect appears to be independent of the nature of the group, for the methyl, chloro, bromo, iodo, nitro, and hydroxyl radicals impede the union with glycine. Substitution in meta and para position, on the other hand, exerts no steric hindrance.

The conjugation of benzoic acid with glycuronic acid is also affected by substitution in ortho position, but the nature of the substituting group is of fundamental importance. Taking the conjugation of benzoic acid as standard, it was found that a neutral group such as the methyl exerted no effect; an acid group like the chloro or the hydroxy caused a definite decrease in the excretion of the glycuronic acid compound; a strongly acid group such as the nitro suppressed the excretion almost completely; whereas a basic group like the amino produced a marked increased output of the glycuronic acid compound.

Excretion of uncombined acid was observed only when a strongly acidic group occupied the ortho position.

TABLE I.

	Excretion of compound (in terms of benzoic acid)							
			Combined with					
Compound	Free		Glycine			Glycuronic acid		
	6 hr.	12 hr.	6 hr.	12 hr.	24 hr.	6 hr.	12 hr.	24 hr.
Benzoic acid	—	—	0.60	0.55	0.21	0.96	0.54	
o-Toluic acid	0.02	—	0.14	0.12	0.13	1.06	0.89	0.41
o-Chloro-benzoic acid	0.36	0.27	0.12	0.11	0.30	0.54	0.50	0.64
o-Bromo-benzoic acid	0.36	0.05	0.09	0.09	0.20	0.58	0.53	0.57
o-Iodo-benzoic acid	0.01	0.02	0.12	0.04	0.09	0.47	0.41	0.40
o-Hydroxy-benzoic acid	0.09	0.06	0.08	0.07	0.17	0.48	0.50	0.80
o-Nitro-benzoic acid	1.32	0.77	0.11	0.08	0.08	0.24	0.18	0.21
o-Amino-benzoic acid						1.67	0.50	
Control			0.11	0.10	0.15	0.20	0.12	0.20

The various compounds were neutralized with sodium hydroxide and mixed with a glycine free diet. The equivalent of 3 gm. of benzoic acid was fed. In the case of o-iodo-benzoic acid only half that amount was given.

The importance of these considerations from the point of view of correlating pharmacological action with chemical structure is illustrated by salicylic acid, a compound containing the acidic hydroxy radical in ortho position to the carboxyl group.

5951

A Protective Property of Serum in Irradiation of Amylase with Ultraviolet Light.

WILLIAM R. THOMPSON AND ROBERT TENNANT.
(Introduced by Raymond Hussey.)
From the Department of Pathology, School of Medicine, Yale University.

The monomolecular character of the inactivation of pepsin by ultraviolet light has been demonstrated.[1] Recently, similar results[2] have been obtained with amylase, employing a viscosimetric method for estimations of concentration. As in the earlier work, infra-red radiation was absorbed in a filter of 5 mm. of water and it was demonstrated that the interposition of a No. 1 Crookes Glass filter prevented passage of the radiation responsible for the changes otherwise observable.

It seemed of interest to ascertain whether or not similar effects could be obtained with amylase in sera. A dilution of about 1 part of dog serum with 4 parts of 0.85% saline (containing 1.56 ± 0.02 units of amylase as estimated viscosimetrically) was irradiated for a period of 3 minutes at 10.0 ± 0.15°C. under conditions such that a simple 0.85% saline solution of pancreatin would have been about half inactivated, according to the findings of the previous work.[2] The estimated amylase concentration after irradiation was 1.52 ± 0.02 units—not significantly different from the previous value (the indicated dispersion measures being the A.D.). In a similar manner solutions containing serum from the same animal mixed in 0.85% saline with pancreatin solution (concentration of amylase, about 10 units) were irradiated with evidence of similar protection in the case of those containing 20% serum even when irradiated under the same conditions for 30 minutes. Finally, such a solution containing but 0.2% serum was about half inactivated in 6 minutes of such irradiation, about half the reaction speed of a similar solution without serum.

Other workers have observed protective effects upon enzymes in the irradiation of animals[3] with ultraviolet and other radiation from a mercury arc in quartz. A protective influence of serum in the irradiation of a bacteria phage,[4] and protection of amylase by proteins from heat inactivation[5] have been observed.

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[1] Hussey, R., and Thompson, W. R., *J. Gen. Physiol.*, 1925, **9**, 217.

[2] Thompson, W. R., and Hussey, R., *J. Gen. Physiol.*, 1931, **15**, 9.

Further work is planned to investigate the nature of such protection as well as its potentiality in other animals of the same and different species.

5952

Influence of Splanchnic Nerves on Gastric Secretion.

STEWART G. BAXTER. (Introduced by B. P. Babkin.)

From the Department of Physiology, McGill University.

Very little is known about the influence of the sympathetic nervous system on the gastric glands (Volborth and Kudryavzeff[1]), and the data concerning the effect of epinephrine on the gastric secretion are rather confusing (Lim,[2] Ivy and McIlvain,[3] Sirotinin,[4] Hess and Hundlach[5]).

Experiments were performed on dogs and cats anesthetized with a mixture of chloralose and urethane. The splanchnic nerves were isolated below the diaphragm and placed on shield electrodes. The vagi were either cut at the neck or tied around the oesophagus below the diaphragm. The pylorus was ligated and a fistula placed in the posterior wall of the stomach and brought out in the left flank through a stab wound.

Rhythmic stimulation of the splanchnics was carried out in this fashion for periods of 8 to 10 hours. In most of the experiments a secretion of thick alkaline mucoid fluid began during the first hour of stimulation and continued at a steady rate thoughout the experiment. In dogs of average weight (8 to 10 kg.) the hourly amount was from 3 to 5 cc., and in cats 1 to 2 cc. Occasionally when the stomach reaction was acid at the beginning of the experiment, the samples of mucus during the first few hours remained faintly acid, but the acidity rapidly diminished and ultimately the reaction became alkaline. When the secretion was acidified (Metts' method) it showed a moderate peptic power (75 to 200 units). The chloride content was lower than that of gastric juice, viz., 380 to 450 mg. Cl.

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[3] Koldayer, B. M., and Altschuller, M. M., *Z. Physiol. Chem.*, 1930, **186**, 223.

[4] McKinley, E. B., Fisher, R., and Holden, M., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 408.

[5] Nakamura, H., *J. Soc. Chem. Ind. Japan*, 1930, **33**, 521, 523, 524.

[1] Volborth, G. W., and Kudryavzeff, N. N., *Am. J. Physiol.*, 1927, **81**, 154.

[2] Lim, R. K. S., *Quart. J. Exp. Physiol.*, 1922, **13**, 79.

[3] Ivy, A. C., and McIlvain, G. B., *Am. J. Physiol.*, 1923, **67**, 124.

[4] Sirotinin, G. W., *Z. f. d. ges. exp. Med.*, 1924, **40**, 90.

[5] Hess, W. R., and Hundlach, R., *Pflüger's Arch.*, 1920, **185**, 122.

In some experiments the pylorus was divided from the body and fundus, and the secretion was collected separately from both parts. This showed that the secretion was not exclusively from the pyloric glands.* The content of total solids and organic material in the secretion diminished progressively, as observed in other glandular structures, indicating that it was a true secretion and not merely an expulsion of surface mucus. Atropin did not abolish the secretion.

In other experiments repeated injections of epinephrine were made (0.5 cc. of a 1/10,000% solution every 5 minutes). The same type of secretion was observed in these experiments and the secretion exhibited the same properties.

These experiments indicate that the sympathetic nervous system has a definite relation to the mucoid elements of the gastric mucosa. Further experimentation is necessary to demonstrate whether the sympathetic nervous system has any relationship to the peptic glands.

5953

Protein Fractions of the Timothy Grass Bacillus.*

MICHAEL HEIDELBERGER AND A. E. O. MENZEL.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

The procedure described recently for the isolation of protein fractions from a scarlatinal strain of *Streptococcus hemolyticus*[1] has been applied with slight modification to the Timothy grass bacillus, *Mycobacterium phlei.*

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* In a personal communication to Dr. B. P. Babkin, Professor B. A. McSwiney of Leeds, England, stated that stimulation of the sympathetic nerve produced a very scant mucoid secretion from the pyloric part of the stomach in decerebrated cats.

* Carried out under a grant from the National Tuberculosis Association and with the aid of the Harkness Research Fund, Presbyterian Hospital, New York.

[1] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1931, **54**, 513.

Fresh cultures were frozen and dried,† extracted in the cold with acetone and purified ether during 48 hours, ground in a ball mill until intact organisms were no longer visible, and again extracted in the cold with acetone and ether during several hours. The cell residues were then stirred for about 6 hours each in the cold with buffer at pH 4 (C), 6.5 (D, D′), water containing enough *N* NH_4OH to keep the pH at 8.3-8.5 (E), water made alkaline to about pH 9 (F), and water made alkaline to about pH 11 (G). The residual material was shaken with 0.5% NaOH at room temperature (K). The properties of the fractions obtained by acidifying the extracts are given below, D′ differing from D only in the necessity for the use of acetone in precipitating it from the opalescent supernatant from D.

TABLE I.

	$[\alpha]_D$ degrees	N %	P %	Basic Ash %	Precipitin reaction of 1:1000 solution with	
					anti-human strain serum†	anti-timothy serum†
D	+14	15.2	3.2	0.9	+	+
D′	+22	16.0	3.8	1.4	+	±
E	—34	17.1	1.6	0.8	+±	+
F	—51	15.8	0.6	—	+	±
G	—47	14.4	0.7	0.5	+	+
K	—38	13.0	0.4	0.4	+	+

It is thus seen that a close parallel exists with the corresponding fractions of the hemolytic streptococcus. In general levorotation increases and nitrogen and phosphorus decrease with increasing alkalinity of the solution used for extraction. The lability of the D fraction is not as great in the case of the Timothy grass bacillus, only 20% N and 60% P being split off in 0.02 *N* NaOH at 25° for 24 hours. Fraction D corresponds to some extent with the "water-soluble protein", K, with the "alkali-soluble protein" obtained according to Johnson's scheme.[2] The reactivity of the fractions toward antihuman type serum is surprising in view of the specificity of the tuberculin proteins.[3]

The work is being continued and the proteins of the human type of tubercle bacillus are now being studied in the same way.

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† Through the courtesy of the H. K. Mulford Biological Laboratories, Glenolden, Pa.

[2] Johnson, T. B., *Am. Rev. Tuberc.*, 1926, **14**, 169.

[3] Seibert, F. B., *Am. Rev. Tuberc.*, 1930, **21**, 370.

5954

A New Method for Staining Bacterial Capsules.*

J. W. CHURCHMAN AND N. V. EMELIANOFF.

From the Laboratory of Experimental Therapeutics, Cornell Medical College.

A simple method is described by which a capsule-like structure is readily demonstrated about many bacteria. It is selectively stained pink, in contrast to the blue bacterial bodies. Use is made only of stain and fixative. No artificial steps, like preliminary treatment with serum, etc., designed to render the capsule more readily demonstrable, are taken.

The technic follows: Smear dried in air, covered with 10 drops of Wright's stain, which is left on until it has nearly but not quite evaporated to dryness when a pinkish color replaces the original blue of the stain. This usually takes about 3 or 4 minutes. Wash off as rapidly as possible with Clark and Lub's buffer pH 6.4 to 6.5. (Sometimes a better specimen is obtained if smear is also washed rapidly with distilled water.) Dry with fan without blotting. If washing with buffer is done before stain has evaporated, the capsule-like structures will not be stained. Excellent results may also be obtained by leaving the air dried smear in Wright's stain over night, removing and allowing to evaporate until a pinkish color is reached, then dipping rapidly through buffer and drying with fan.

If technic be carried out correctly the capsule-like structure stains as a pink area surrounding the blue bacteria and itself limited by a definite membranous pink-stained periphery, on the outer surface of which precipitated stain is sometimes observed. Outside this structure a clear zone is occasionally (as in *Diplococcus pneumoniae*) seen. If staining be too deep the whole organism (capsule-like structure and bacterial body) is stained purplish and no distinction can then be made out between the parts. If staining is not deep enough or washing too prolonged all stain is removed from the capsule-like structure, only the blue bacterial bodies being then visible.

Three known capsulated organisms have been studied by this technic [*Diplococcus pneumoniae* types 1, 2, and 3; *B. anthracis* Koch (6 strains); *Klebsiella pneumoniae friedländer* (7 strains)] and the capsule-like structure clearly demonstrated. It occupies the site and has the characteristic of the capsule as demonstrated by the usual capsular stains. It is as readily demonstrated on "R" strains of pneumococcus as on "S" strains.

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* Work aided by grant from the Chemical Foundation.

This structure may be made apparently to disappear from *B. anthracis* by suspending the organisms in 1% NaCl and then staining. It reappears however if the organisms are washed free of the salt and restained.

The unfortunate circumlocution "capsule-like structure" has been employed not because the findings are ambiguous but because the capsule has never been accurately defined and the confusion to which the use of terms like "pseudo-capsule", "aureole", "clear zone", etc., has led make it impossible to speak more scientifically. There seems little doubt however that the structure stained by this method is the true capsule.

Two minor defects of this method should be mentioned. Precipitation of stain sometimes produces a granular deposit which makes the background confusing. Experiments now going on indicate that it may prove possible to do away with this disadvantage. Evaporation of Wright's stain also sometimes results in the formation of ringlike bodies which somewhat resemble capsules, although careful study shows them to be artefacts. Though occasionally causing confusion these bodies are usually easily to be distinguished from the true stained capsules.

5955

Presence of Capsules on "Non-Capsulated" Microorganisms.*

J. W. CHURCHMAN AND N. V. EMELIANOFF.

From the Laboratory of Experimental Therapeutics, Cornell Medical College.

That capsules may be rather widely distributed throughout the bacterial field has been frequently suggested. Streptococci with capsules were reported by Tavel and Krumbein[1] (*cf.* the "*streptocoque auréolé*" of LeRoy de Barres and Weinberg[2]) and Hiss[3] convinced himself that many if not all strains of *Streptococcus pyogenes* are capsulated. Boni[4] claimed to have demonstrated the presence of these structures on many different types of organisms. However his method of covering the smears (before staining) with egg albumin and glycerine hardly provided conditions in which artefacts could be excluded, and this fact has thrown considerable doubt on the inference he drew. Still, the statement is often encountered in the literature that many, if not all, varieties of bacteria *may* be capsulated; a statement which is correctly described as a guess. Huntoon is generally known to have thought that he had demonstrated capsules on several varieties of "noncapsulated" bacteria. His only published statement, however, appears to be the following, written in connection with the description of the capsular stain with which his name is connected[5]: "This method has been tried on streptococci, staphylococci, members of the Gram negative group and many flagellate bacilli and has in no instance shown a capsule on these organisms though a similar structure may be demonstrated on all by means of a special technic which will be the subject of a subsequent report."

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* Work aided by a grant from the Chemical Foundation.

[1] Tavel and Krumbein, *Centralbl. f. Bakt. Ref.*, 1895, **18**, 547.

[2] de Barres and Weinberg, *Archiv. de med. et d'anat. pathol.*, 1899, **11**, 399.

[3] Hiss, *J. Exp. Med.*, 1905, **6**.

[4] Boni, *Centralbl. f. Bakt.*, 1900, **28**, 705.

By the technique described in the previous communication we have examined smears from agar cultures of the following "noncapsulated" organisms and have found them to be surrounded by a definite structure, limited by a membranous periphery selectively stained pink, which is similar to the capsule-like structure demonstrable about *Diplococcus pneumoniae* by the same staining method.

	Strains
1. *Serratia marescens* Bizio (*B. prodigiosus*)	4
2. *Pseudomonas aeruginosa* (*B. pyocyaneus*)	2
3. *B. subtilis* Ehrenberg Cohn	2
4. *B. subtilis* Chester	1
5. *Escherichia coli*	3
6. *Erysipelothrix murisepticae*	1
7. *Eberthella typhi*	1
8. R strains of *Diplococcus pneumoniae*	6

(2 each of types 1, 2, and 3)

We have also examined a number of specimens of the acid fast group. The findings in a few instances strongly suggest that some members of this group too may possess capsules, but the evidence is as yet too small to warrant definite statements.

All organisms were positively identified in accordance with Bergey's Manual except that no pyocyanin could be definitely obtained from the otherwise typical *pseudomonas aeruginosa.*

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[5] Huntoon, *J. Bact.*, 1917, 2, 241.